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The Role of Native Ecosystems in Constraining Alien Tree Invasions

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THE ROLE OF NATIVE ECOSYSTEMS IN CONSTRAINING ALIEN TREE INVASIONS

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The role of native ecosystems in constraining alien tree invasions
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ABSTRACT

Alien tree invasions cause widespread impacts to ecosystem structure and function in many regions across the globe. Limiting the spread of these invasions into untransformed native ecosystems is often a management priority. Understanding the mechanisms by which these native ecosystems are able to resist tree invasion can not only enhance our understanding of invasion processes but may also aid in predicting invasion risk and informing management decisions on monitoring, removal and restoration. Australian *Acacia* species are particularly invasive in the uniquely biodiverse Cape Floristic Region of South Africa. The generalist reproductive biology of these species results in the ability to reproduce and spread widely in invaded regions. However, little is understood of the ability of native ecosystems to constrain the establishment and growth of these tree invaders. I thus investigated the ability of an untransformed South African shrubland to resist the establishment and growth of an invasive tree species, *Acacia cyclops*, in order to gain a mechanistic understanding of ecosystem resistance. My results indicate that herbivory by an abundant indigenous rodent resulted in mortality of 60-100 % of transplanted *A. cyclops* seedlings. Rodent behaviour, rather than densities influenced herbivory levels since rodents were less likely to forage in sparsely vegetated sandy areas. A separate experiment found that belowground competition reduced *A. cyclops* seedling survival by *ca.* 1.4–1.8-fold and biomass accumulation by 4–5-fold. A review of available evidence from published literature led me to conclude that if able to navigate early constraints and establish ecophysiological traits such as heteroblasty, N_2-fixation and strong allocation to belowground biomass aid invasive Australian *Acacias* to overcome abiotic constraints. These findings indicate that native ecosystems constrain the survival and growth of an invasive tree species. Mechanisms of resistance may be useful in predicting invasion risks to different native communities, identifying targets for ecosystem restoration and informing decisions on management and clearing operations so as to maximize ecosystem resistance.

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TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

CHAPTER 1
INTRODUCTION

1.1 Global tree invasions and management

Alien tree species have been identified to cause some of the most widespread and damaging invasions across the globe (Richardson and Rejmánek 2011). Their large size and long lifespans often result in ecosystem domination leading to reductions in biodiversity, alterations in disturbance regimes, changes to biogeochemical cycling and reductions in available surface water (reviewed in Le Maitre *et al.* 2011, Richardson and Rejmánek 2011). Many of these tree species however, have also come to be valued for multiple uses by local populations, making management and removal highly controversial in social and economic contexts (Carruthers *et al*. 2011, Dickie *et al*. 2014, Kull *et al*. 2011). For this reason and due to the often prohibitive costs and efforts needed to remove established tree invasions (Marais *et al.* 2004) complete eradication is unlikely and managers are instead moving towards reducing invasion spread (Caplat *et al*. 2012; Hulme 2012; Moore *et al.* 2011; van Wilgen *et al.* 2011). Limiting the spread of invasions into untransformed habitats is a management priority since these areas are important for biodiversity conservation and ecosystem functioning in landscapes that are increasingly impacted by human modifications. Understanding the mechanisms of ecosystem resistance to invasion can aid in predicting invasion risk and inform management decisions on monitoring, removal and restoration (Rew *et al*. 2007).

1.2 Ecosystem resistance to tree invasion

Resistance to invasion involves process and properties of native ecosystems that impose barriers to invasion success (Elton 1958). These barriers include both abiotic components such as climate, soil properties, topographic variation and microsite availability as well as biotic barriers, such as plant competition, herbivory, pests and pathogens and a lack of symbionts, pollinators or dispersal agents (Lonsdale 1999, Meier *et al*. 2010, Theoharides and Dukes 2007). While mechanisms of these barriers are recognized to depend both on the ecosystem in question as well as the traits of the invader (Richardson and Pyšek 2006), they are also likely to differ with each stage of invasion linked to the lifecycle of the invader namely: flowering and pollination, dispersal and seed bank accumulation, germination, establishment and growth (Fig. 1.1). Understanding the barriers associated with each step of an invasive tree life cycle and how trees overcome these barriers can provide a mechanistic understanding of ecosystem resistance.

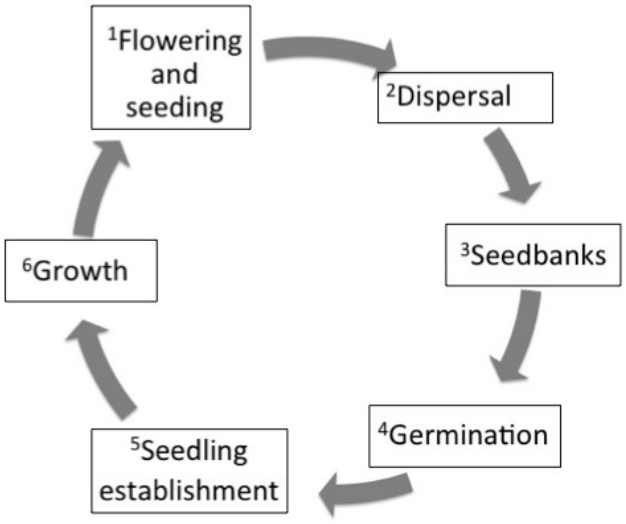

Figure 1.1. Generalized life cycle stages susceptible to ecosystem resistance for an invasive tree species.

1.3 Australian *Acacia* invasions

Australian *Acacias,* of the subgenus Phyllodinae, are the most widely invasive tree genera, with at least 23 species known to be invasive in many regions across the globe (Richardson and Rejmánek 2011). Their impacts are possibly greatest in highly invaded treeless or sparsely wooded ecosystems, which include Mediterranean-climate shrublands in Chile, California (USA), Spain, Portugal and South Africa (Richardson *et al.* 2011, Rundel *et al.* 2014). The Cape Floristic Region (CFR) of South Africa, a biodiversity hotspot and centre of endemism (Goldblatt 1997), hosts the most invasive Australian *Acacia* species of all invaded regions (as defined in Richardson *et al*. 2011), which is particularly significant when considering its conservation importance.

Much research has focused on impacts (reviewed in Le Maitre *et al*. 2011) and management (reviewed in Moore *et al*. 2011, van Wilgen *et al*. 2011, Wilson *et al*. 2011) of these invasive trees. In addition, many studies have contributed to a thorough understanding of the life stages from flowering to germination (Stages 1:4 in Fig 1.1) summarized in a comprehensive review by Gibson *et al.* (2011). The flowering of Australian *Acacias* are unlikely to be constrained by a lack of pollinators, since these trees are pollinated by generalist pollinators such as the widely introduced honeybee (Gibson *et al*. 2011). Australian *Acacias* are known to produce 500-12000 seeds m^{-2} canopy annually in invaded ranges (reviewed in Gibson *et al*. 2011). The seeds are attached to nutrient rich arils that are generally adapted for ant or bird dispersal. In introduced ranges, birds, ants, rodents, baboon, livestock and even humans are reported as dispersal vectors. Seeds have a hard, water impermeable seed coat and can remain dormant in the seedbank for 50-100 years or more and retain a seed viability of up to 99% (Gibson *et al.* 2011). While many of

these seeds are stimulated to germinate by fire, some can be stimulated to germinate through chemical scarification associated with ingestion by dispersal agents (Gibson *et al.* 2011). The generalist reproductive biology of introduced invasive Acacias is thus unlikely to limit invasion spread. In contrast, little is understood of the constraints imposed by native ecosystems to seedling establishment and growth of these widely invasive trees.

1.4 Research outline

In this dissertation, I investigated the resistance imposed by a native Mediterranean-climate shrubland to the establishment and growth (stage 5 and 6 Fig 1.1) of an invasive Australian *Acacia*. Specifically, I conducted a series of field experiments on *A. cyclops,* one of the most widely invasive species in the CFR region. In Chapter 2, I first assess the effects of resource limitation through competition on seedling establishment of *A. cyclops*. Chapter 3, continues to assess constraints to *A. cyclops* seedling establishment by investigating the role of herbivory on seedling survival. In chapter 4, I synthesize the current understanding on resource constraints to growth of invasive Australian *Acacias*, and in Chapter 5, I assess the ecophysiological traits that enable adult A. cyclops trees to overcome resource constraints while competing with native species.

CHAPTER 2:
COMPETITIVE RESISTANCE OF A NATIVE SHRUBLAND COMMUNITY TO INVASION BY THE NON-NATIVE TREE SPECIES, *ACACIA CYCLOPS*

2.1 Abstract

Competitive resistance of native vegetation may constrain the establishment of invasive tree seedlings thereby acting as an important bottleneck to invasion success. This study assessed the effects of aboveground and belowground competition on the survival and early performance of *Acacia cyclops* seedlings in a Mediterranean-climate shrubland through manipulations to light availability, rooting competition and soil fertility.

Aboveground competition constrained seedling survival equally across all light availabilities but belowground competition resulted in 1.4–1.8-fold lower survival. Fertilization had no effect on seedling mortality, suggesting that survival was not driven by competition for soil nutrients. Seedling height was *ca.* 10-35% greater in fertilized seedlings and 50-70% greater in root exclusion seedlings while no difference was seen in heights across light availabilities. Belowground competition reduced seedling height and biomass *ca.* 4–5-fold, whereas greatest levels of competition for light only reduced seedling height by *ca.* 2–4-fold and had no effect on seedling biomass. In unfertilized conditions, seedlings consistently exhibited greater nodule biomass (0.03-0.11 g nodule mass g^{-1} plant mass) compared to fertilized seedlings (*ca*. 0 g nodule mass g^{-1} plant mass) suggesting that *A. cyclops* seedlings may have greater reliance on N_2-fixation under low nutrient conditions. Results provide evidence for the importance of maintaining intact undisturbed native ecosystems that are able to constrain invasion establishment and slow invasion spread.

2.2 Introduction

Tree invasions are responsible for some of the most significant impacts to ecosystem structure and function across the globe and many species are now included in lists of the worlds top invaders (Richardson and Rejmánek 2011). Impacts of tree invasions are often most pronounced in formerly sparsely wooded environments, including Mediterranean-climate ecosystems (Rundel *et al.* 2014) where the dramatic changes in vegetation structure have lead to increased water use, alterations of disturbance regimes and reductions in the diverse and often highly endemic native biodiversity (Gaertner *et al*. 2009; Le Maitre *et al*. 2011; Richardson *et al*. 2014; Rundel *et al*. 2014). These impacts are in part associated with the greater size of the invaders in comparison to native species which confers greater competitive ability for available resources (Richardson and Bond 1991; Rundel *et al.* 2014). As seedlings however, competition from native vegetation may constrain the survival and early growth of invasive tree species, potentially imposing an important bottleneck to invasion success and associated impacts.

Water stress is considered one of the greatest causes of seedling mortality (Moles and Westoby 2002). In Mediterranean-climate ecosystems where low summer rainfalls and drought conditions constrain seedling survival (Frazer and Davis 1988, Lamont *et al.* 1989, Lamont *et al.* 1991, Enright and Lamont 1992), competition for water resources would further limit water availability to seedlings, compounding the likelihood of mortality (reviewed in Vilà and Sardans 1999). Similarly, low soil nutrients, a key feature of many Mediterranean climate regions (Kruger *et al*. 1983, Specht and Moll 1983), are suggested to limit plant growth (reviewed in Sardans *et al*. 2006; Vilà and Sardans 1999). Competition for soil nutrients has been shown to occur even under these low soil nutrient conditions,

although competitive effects are suggested to be more intense when soil nutrients are less limiting (reviewed in Vilà and Sardans 1999). Competition for scarce soil nutrients may thus further constrain seedling establishment. Increased competition for light under vegetation canopies can also negatively impact seedling performance by decreasing growth and survival rates (reviewed in Vilà and Sardans 1999). In Mediterranean climate shrublands, aboveground competition can often be variable, related to the patchy nature of vegetation clumps interspersed with areas of less dense vegetation or open patches (e.g. Abanda *et al.* 2011) and competition for light availability is thus likely to vary spatially.

In the nutrient poor sclerophyllous shrublands of the Mediterranean-climate Cape Floristic Region (CFR) of South Africa several tree species have become widely invasive (Henderson 2007). *Acacia cyclops* A. Cunn. Ex. Don (Fabaceae), originally introduced for dune stabilization from the mid 1800's through the early 1900s (Poynton 2009), is one of the most widespread and abundant invasive Australian acacias in the region (Henderson 2007). Dense monospecific stands are often associated with disturbance, but the ability of *A. cyclops* to also colonize areas of intact native vegetation is of great concern (Stirton 1980, Richardson *et al*. 1997). The spread of this species into native vegetation is largely attributed to seed dispersal by native bird species (reviewed in Gibson *et al*. 2011, Glyphis *et al.* 1981, Fraser 1990). The growth and competitive ability of *A. cyclops* is suggested to be supported by several key traits that include: substantial allocation to rooting biomass and depth, strong N_2-fixation abilities as well as heteroblasty which is suggested to confer high relative growth rates as bipinnate seedlings but long-lived, nutrient-conserving tissues as phyllodinous adults (reviewed in Morris *et al*. 2011). The persistent spread of invasive propagules into native vegetation coupled with the competitive traits of the invader,

provides an ideal context for which to investigate the dynamics of competitive resistance on seedling survival and growth of an invasive tree species.

In this study I aimed to assess the capacity for native competition to constrain the survival and early growth of an invasive tree species, *A. cyclops*, in the nutrient-poor, Mediterranean-climate strandveld vegetation community of the Cape Floristic Region, South Africa. I hypothesized that belowground competition for water and nutrients would decrease both seedling survival and seedling performance whereas aboveground competition from light would have greater effects on seedling performance rather than seedling survival. I assessed the competitive effects of native vegetation on *A. cyclops* seedlings through a series of field manipulation studies.

2.3. Methods

2.3.1. Study sites

Research sites were located in Koeberg Nature Reserve (33.65287 S, 18.43725 E) and Vergaderingskop Nature Conservancy (34.55466 S, 19.37298 E) in the Western Cape Province of South Africa. At least 60% of rainfall occurs in the cooler winter months with mean annual precipitation ranging between 500 – 533 mm. Mean annual temperatures are 16 - 17°C, with daily temperatures ranging between 7 – 26°C (average monthly minima – maxima).

The sites are located in strandveld coastal vegetation. Koeberg is classified as Cape Flats Dune Strandveld, while Vergaderingskop is considered Blombos Strandveld (Mucina and Rutherford 2006). Strandveld vegetation is 1-2 m in height and is dominated by broad-leaved, sclerophyllous shrubs interspersed with succulents, bulbs and grasses (Mucina &

Rutherford 2006). Invasion of *A. cyclops* within strandveld is patchy with densely invaded sites occurring in some areas, while others sites appear to be relatively uninvaded. Disturbance through fire is not as common in strandveld systems in comparison to the surrounding CFR vegetation types, and fire return intervals are estimated to be 50-200 years (Rebelo *et al*. 2006). The soils of strandveld are deep, well drained marine-derived aeolian sands, rich in calcium, making the soils neutral to alkaline. While soil Ca and total P are relatively high compared to other vegetation types of the CFR, total N, available P, K, Na and Mg are comparably low (Table 2.1).

Table 2.1 Soil nutrient concentrations (mean ± S.E.) at Koeberg and Vegaderingskop (n=20) strandveld study sites. Soil nutrient characteristics from other CFR vegetation types are presented for comparison (Cramer *et al*. in press, Stock *et al*. 1995).

	Strandveld		Fynbos	Renosterveld
	Koeberg n=17	Vergaderingskop n= 20		
Total N (mg kg^{-1})	455 ± 54	1443 ± 124	1700 ± 100	1200 ± 100
Total P (mg kg^{-1})	959 ± 13	507 ± 23	249 ± 10	279 ± 45
Available P (Bray II; mg kg^{-1})	11.5 ± 0.5	10.2 ± 0.8	13.3 ± 1.7	13.3 ± 1.2
K (mg kg^{-1})	19 ± 1	73 ± 6	84 ± 5	143 ± 16
Na ($cmol^{+}$ kg^{-1})	0.09 ± 0.003	0.35 ± 0.3	0.22 ± 0.03	0.35 ± 0.04
Ca ($cmol^{+}$ kg^{-1})	12.4 ± 0.27	16.7 ± 0.59	3.7 ± 0.3	5.2 ± 1
Mg ($cmol^{+}$ kg^{-1})	0.37 ± 0.02	1.6 ± 0.16	1.3 ± 0.1	2.3 ± 0.4
$\delta^{15}N$ (‰)	1.4 ± 0.3	4.9 ± 0.2	4.7 ± 0.4	5.6 ± 0.2

2.3.2. Experimental design

To examine the effects of competition from established native vegetation on survival and performance of *A. cyclops* seedlings, I manipulated aboveground competition, belowground competition as well as soil nutrient availability in a factorial design. Five plots (*ca.* 60 x 60 m) were established at each site and within each plot four subplots (*ca.* 15 x 15 m) at Koeberg (20 total replicates) and three subplots at Vergaderingskop (15 total

replicates) were established 20-40 m away from each other (Fig. 2.1). The lower number of subplots at Vergaderingskop was due to the remote location of this site, which resulting in limited capacity to transport research materials.

At each experimental subplot, ten pairs of *A. cyclops* seedlings (0.5 m apart) were planted under five categories of light availability (two pairs at 0-20%, 20-40%, 40-60%, 60-80% and 80-100% respectively). Seedlings were allotted to a light category based on the amount of light diffusion through vegetation canopy measured using a LI-COR LAI-2000 ceptometer with the sensor placed at seedling height. One seedling of each pair was planted within a PVC root exclusion tube 0.2 m diameter x 0.4 m long (Fig. 2.2) to separate roots of *A. cyclops* seedlings from root competition of surrounding native vegetation. The PVC tubes were driven into the soil *ca*. two months before seedlings were planted to diminish disturbance effects on seedling growth. The exclusion tubes ere also weeded of other vegetation throughout the duration of the study. One set of seedling pairs in each light category were treated with 50 g N, 10 g P, 40 g K, 3.5 g Mg per seedling through slow-release fertilizer addition (Haifa group: Multicote [8] 15N-3P-12K+Mg+trace elements), placed in a 10 cm radius away from the seedlings, place directly into the soil at a depth of *ca.* 5-10 cm. Slow release fertilizer was used to provide increased nutrient availability throughout the growth period rather than one initial pulse. The seedlings were protected from vertebrate herbivory by enclosure in 0.2 m diameter x 0.3 m high diamond mesh (1.3 cm mesh) cages (Fig. 2.2).

Acacia cyclops seedlings were grown from seeds collected at Vergaderingskop. Seeds were scarified with sandpaper and grown in trays of sand/potting soil mix in the University of Cape Town Botany greenhouse in June 2012. Seeds were watered daily until

germination occurred. After germination, seedlings were watered every second day in order to harden them in preparation for transfer to the field experiment. On appearance of the second set of bipinnate leaflets, approximately 14 days after initial sowing, seedlings were transplanted into the field experiment. Transplanting took place on 26-27 June 2012 at Koeberg and 10-11 July 2012 at Vergaderingskop. Seedlings were watered every second day for 10 days and any individuals that died during this time were replaced. Seedling survival was assessed 40 days later. Of the total seedlings, 28% died within 40 days of planting and this mortality was not correlated to treatments of root exclosures, fertilization, or light availability. Mortality during this period was thus assumed to be due to transplant shock and these seedlings were excluded from analyses.

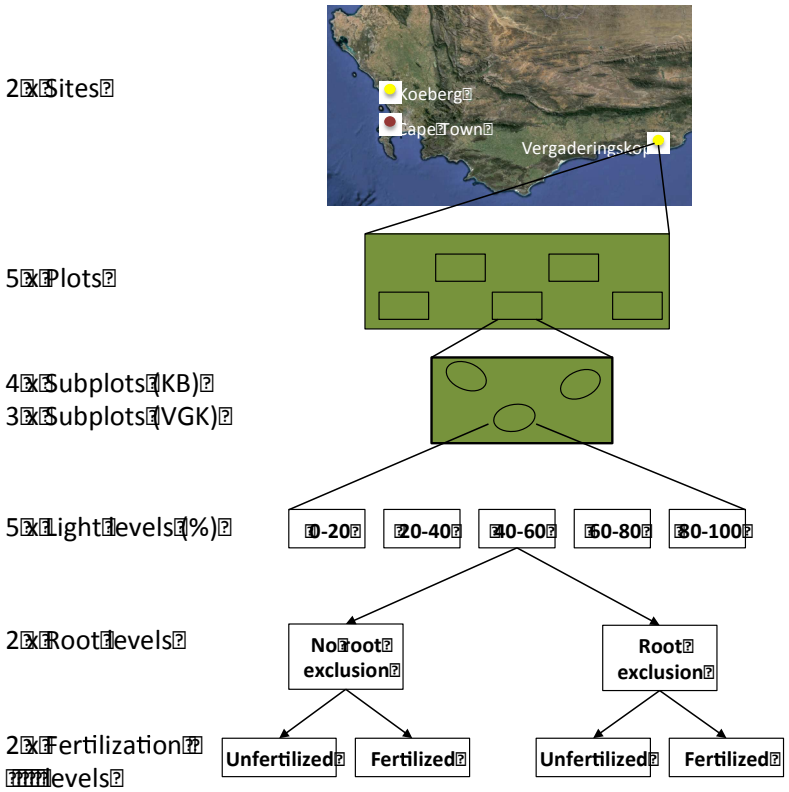

Figure 2.1 Graphic representation of experimental design. Two sites were sampled (KB: Koeberg and VGK: Vergaderingskop) and at each site 5 plots with 3-4 subplots were selected. In each subplot, 20 *Acacia cyclops* seeding were planted according to the three-way interaction of the treatments of light, rooting, and fertilization levels.

Figure 2.2 Photograph showing the experimental setup of *A. cyclops* seedlings planted within a root exclusion tube (left) and without (right) protected by mesh cages.

2.3.3. Seedling measurements

After six months of growth, seedling survival and seedling performance were assessed. Seedling height, biomass gain and foliar N concentrations were used as indicators of plant performance. Foliar $\delta^{15}N$ values were assessed as an indication of N_2 fixation and foliar $\delta^{13}C$ was used to assess integrated lifetime stomatal activity of the seedlings since $\delta^{13}C$ is correlated with the ratio of CO_2 concentration in the intercellular space of leaves to the CO_2 in the atmosphere (C_i/C_a; Farquhar *et al.* 1982)

Aboveground biomass was clipped at the soil surface and belowground biomass (roots) of the seedlings were excavated from within the root exclusion tubes. For seedlings not planted in root exclusion tubes, rooting systems were followed and excavated from a similar soil volume as the root exclusion tubes as best as possible. N_2-fixing nodules were collected off roots as indicators of N_2-fixation potential. All plant material was dried at 70° C for 48 h and weighed.

Foliar N, foliar $\delta^{15}N$ and foliar δ^{13} were measured using mass spectrometry (Department of Archeometry, University of Cape Town, South Africa). *Acacia cyclops* seedlings exhibit two leaf forms in early stages of growth (bipinnate leaflets associated with germination and thereafter modified flattened petioles or 'phyllodes'). Since some seedlings had already lost all bipinnate leaves, only phyllodes were used for foliar tissue analysis. Phyllodes were ground in a Wiley mill to pass through a 1 mm sieve. Milled samples were weighed into tin capsules and combusted in a Thermo Flash EA 112 series elemental analyzer coupled with a Delta Plus XP isotope ratio mass spectrometer (Thermo Electron Corporation, Milan, Italy). An International Atomic Energy Authority standard was used to calibrate results.

2.3.4. Soil analysis

Surface soil cores (0-10 cm deep x 8 cm diameter) were collected at the end of the study (January 2013) from twenty transplant locations selected from across subplots where seedlings had died from transplant shock so that soil nutrient concentrations were representative of potentially available nutrients independent of plant uptake. Cores were taken from both inside and outside root exclusion tubes of both fertilized and unfertilized soils. Soils were air dried for 72 h and passed through a 1-mm sieve to remove rocks and course organic matter. Soil nutrient concentrations of total N, NH_4^+, NO_3^-, available P (Olsen), and K, were analyzed by the Institute for Plant Production (Department of Agriculture: Western Cape, South Africa) following standard protocols (Soil Science Society of South Africa 1990).

2.3.5. Data analysis

I used linear mixed effects models to assess treatment affects on plant performance variables using the function *lmer* in the *lme4* package (Bates *et al.* 2013) in R. (R Core Team 2012). For analysis of belowground treatment effects on soil nutrient variables, models were specified with fixed effects as a three-way interaction between site (Koeberg and Vergaderingskop), root treatment (no exclusion vs. root exclusion) and fertilization treatment (unfertilized vs. fertilized), while subplot was specified as a random effect. For analysis of treatment effects on seedling survival and performance, models were specified with fixed effects as a four-way interaction between site, light availability (0-20%, 20-40%, 40-60%, 60-80% and 80-100%), root treatment (no exclusion vs. root exclusion) and fertilization treatment (unfertilized vs. fertilized), while subplot was specified as the random effect. Data were log transformed before analysis if assumptions of normality were not met.

Best-fit models were derived by the deletion of fixed effect variables one at a time from the full model as described in Buckley *et al.* (2003). Significance of variables retained in the final best-fit model were determined using the Wald $\chi 2$ statistic with the *Anova* function in the Applied Econometrics with R (AER) package (Kleiber and Zeileis 2008). Post-hoc differences were determined by comparing contrasts of least-squares means using the *lsmeans* function in the package *lsmeans* (Lenth 2014). Plots of fitted and observed values and residuals were examined to ensure deviations from homoscedasticity and normality did not occur as described by Pinheiro and Bates (2000).

2.4. Results

2.4.1. Soil fertilization

Prior to fertilization, soil nutrient pools were higher at Vergaderingskop than Koeberg for all soil nutrients except NH_4^+ (Table 2.2, Table 2.3). Total N was *ca.* 5.5-fold greater, NO_3^- *ca.* 2.4-fold greater, available P was *ca.* 1.6-fold greater and K was *ca.* 4.5-fold greater at Vergaderingskop than at Koeberg (Table 2.3). At Koeberg, fertilization resulted in a 1.5-fold increase in soil NH_4^+, while NO_3^- increased *ca.* 70-fold. Available P increased *ca.* 1.5-fold and K increased *ca.* 5-fold (Table 2.2, Table 2.3). At Vergaderingskop, fertilization increased all measured soil nutrient concentrations except for total N. Soil NH_4^+ and NO_3^- increased by *ca.* 8-fold and 80-fold respectively. Available P increased by *ca.* 2.4-fold and K by *ca.* 5-fold (Table 2.2, Table 2.3). This suggests that fertilization likely maintained higher nutrient availability to fertilized seedlings. Additionally, root exclusion tubes did not appear to have an affect on soil nutrient availabilities (Table 2.2).

Table 2.2. Wald $\chi 2$ test statistics and significance for fixed effect factors retained in best fit linear mixed effects models of soil nutrient concentrations. Full models were specified with site (S), fertilizer treatment (F) and root treatment (R) as fixed effects and subplot as the random effect.

	Total N	NH_4^+	NO_3^-	Available P	K
Fixed effects	$\chi 2$	$\chi 2$	$\chi 2$	$\chi 2$	$\chi 2$
S	25.0 ***	9.9**	17.0***	42.1***	250.0***
F	17.1***	17.8***	403.5***	102.7***	108.4***
R	*NS*	*NS*	*NS*	*NS*	*NS*
S × F	6.5*	11.4***	NS	31.4***	NS
S × R	*NS*	*NS*	*NS*	*NS*	*NS*
F × R	*NS*	*NS*	*NS*	*NS*	*NS*
S ×F × R	*NS*	*NS*	*NS*	*NS*	*NS*

*** $P < 0.001$, ** $P < 0.01$, * $P < 0.05$, $^+$ $P < 0.1$. *NS* = No significance

Table 2.3. Soil nutrient concentrations (mean ± S.E.) of unfertilized and fertilized surface soils at each site, measured six months after slow release fertilizer addition. Significant differences between soil nutrient concentrations are indicated with lower-case lettering ($P < 0.05$).

Soil nutrient (mg kg^{-1})	n	Koeberg		Vergaderingskop	
		Unfertilized	Fertilized	Unfertilized	Fertilized
N	10	236 ± 76^{a}	351 ± 82^{b}	1327 ± 157^{c}	1542 ± 221^{c}
NH_4^+	6	2.0 ± 0.1^{a}	3.0 ± 0.2^{a}	3.0 ± 0.1^{a}	24.2 ± 8.1^{b}
NO_3^-	6	0.32 ± 0.05^{a}	22.8 ± 6.7^{b}	0.77 ± 0.09^{c}	61.4 ± 9.4^{d}
Available P (Citric acid)	10	100 ± 8^{a}	111 ± 5^{a}	69 ± 5^{b}	103 ± 6^{c}
Available P (Olsen)	10	2.4 ± 0.2^{a}	3.5 ± 0.3^{b}	4 ± 0.4^{b}	9.5 ± 0.5^{c}
K	10	10 ± 1^{a}	47 ± 7^{b}	45 ± 2^{b}	204 ± 20^{c}

2.4.2. Seedling survival

Seedling survival differed between sites and on average was *ca.* 1.5-fold greater at Vergaderingskop (61 ± 3.6 %) than at Koeberg (42 ± 4.1 %). Control seedlings (unfertilized with no root exclusion tubes) had more than double the survivorship at Vergaderingskop than at Koeberg (*ca.* 50% and 20% respectively; Table 2.4, Fig. 2.3). Within sites, light availability and fertilization did not affect seedling survival (Table 2.4) but seedlings in root exclusion tubes had *ca.* 1.8-fold greater survival at Koeberg, and *ca.* 1.4-fold greater survival at Vergaderingskop compared to seedlings not in root exclusion tubes (Table 2.4, Fig. 2.3).

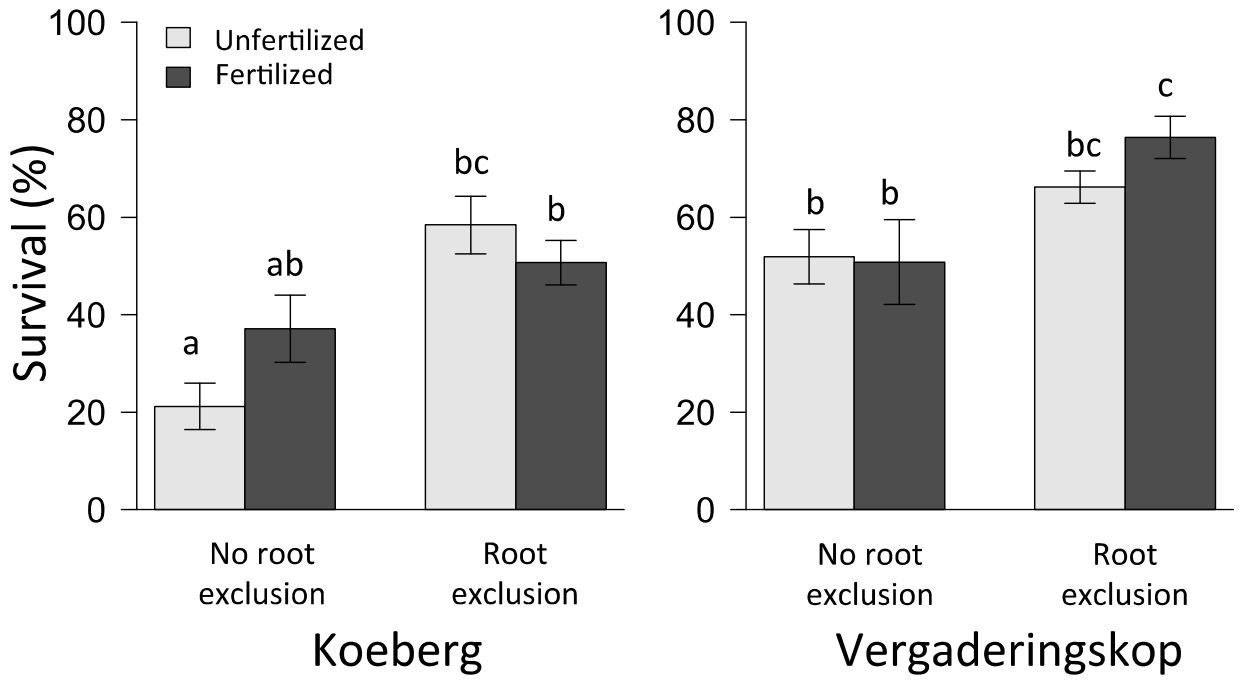

Figure 2.3. Survival of unfertilized and fertilized *A. cyclops* seedlings without and with root exclusion tubes, six months after planting, at Koeberg and Vergaderingskop study sites. Bars represent the mean ± SE and dissimilar letters above bars indicate significant differences ($P < 0.05$) between treatments determined by comparing contrasts of least-squares means.

2.4.3. Seedling performance

Seedling heights were on average *ca.* 50% greater at Vergaderingskop (13.2 ± 0.7 cm) than at Koeberg (8.8 ± 0.3 cm; Table 2.4, Fig. 2.4). Within sites, light availability did not influence final height of seedlings, whereas both fertilization and root exclusion tubes yielded increased seedling heights (Table 2.4, Fig. 2.4). At Koeberg, fertilization resulted in a *ca.* 10% average increase in overall seedling height, while root exclusion resulted in a *ca.* 50% height increase. At Vergaderingskop, fertilization increased seedling height by *ca.* 35%, while root exclusion resulted in a *ca.* 70% increase in height (Fig. 2.4).

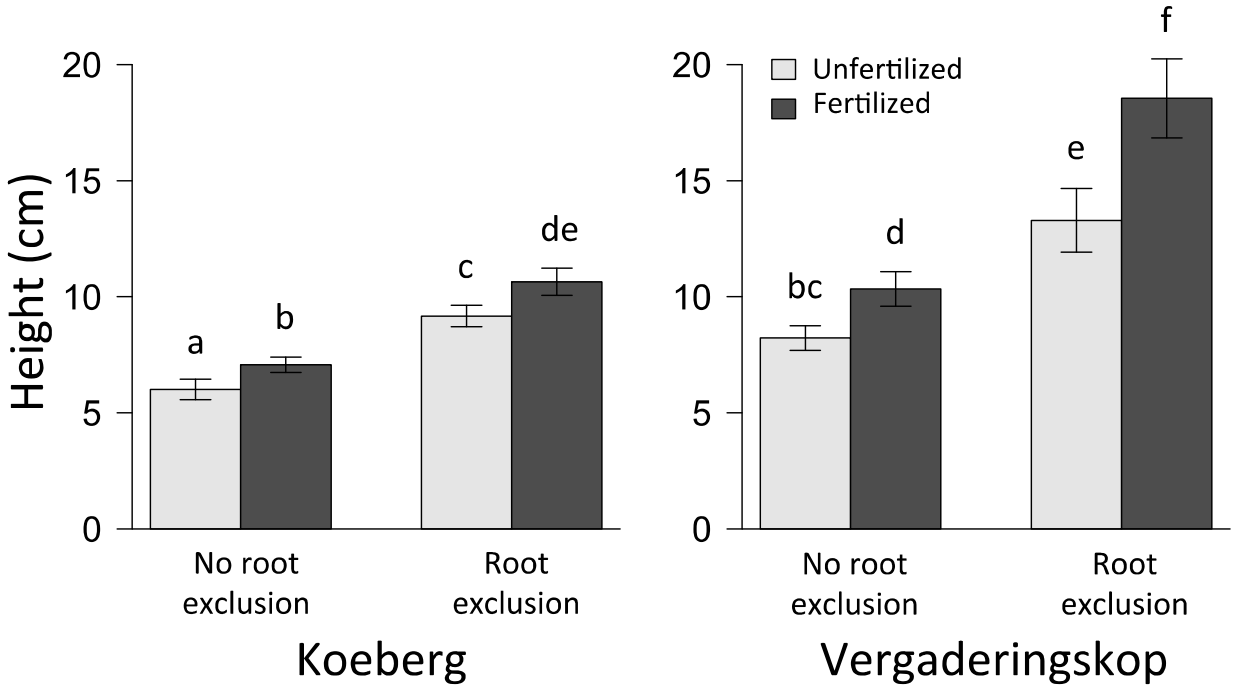

Figure 2.4. Final height of unfertilized and fertilized *A. cyclops* seedlings without and with root exclusion tubes after six months of growth at Koeberg and Vergaderingskop study sites. Bars represent the mean ± SE and dissimilar letters above bars indicate significant differences ($P < 0.05$) between treatments determined by comparing contrasts of least-squares means.

Table 2.4. Wald $\chi 2$ test of significant fixed effects for the best-fit model of *A. cyclops* seeding survival and performance response variables. Full interaction models were specified with site (S), light availability (L), fertilizer treatment (F) and root treatment (R) as fixed effects with subplot specified as the random effect. Fixed effect variables that did not significantly effect predictions of the response variable were removed from the best-fit model and are thus indicated as not significant (*NS*).

Fixed effects	Survival	Height	Aboveground biomass	Belowground biomass	Total biomass	Shoot:root	$\delta^{13}C$	Foliar N	Nodule mass: total biomass	$\delta^{15}N$
S	14.6***	17.9***	*NS*	10.5**	*NS*	18.4***	323***	25.4***	13.1***	46.6***
L	*NS*	*NS*	16.4**	16.9**	17.9**	*NS*	*NS*	*NS*	*NS*	*NS*
F	*NS*	15.4***	49.8***	22.4***	48.22***	19.2***	123***	54.8***	182.4***	*NS*
R	34.8***	62.4***	89.9***	104.5***	106.5***	*NS*	4.3*	42.3***	3.9*	4.2*
S × L	*NS*	*NS*	*NS*	*NS*	*NS*	*NS*	*NS*	*NS*	*NS*	*NS*
S × F	*NS*	*NS*	*NS*	*NS*	*NS*	4.6*	9.0**	*NS*	18.4***	*NS*
S × R	*NS*	*NS*	*NS*	*NS*	*NS*	7.4**	*NS*	*NS*	*NS*	4.0*
F × R	*NS*	*NS*	*NS*	*NS*	*NS*	*NS*	6.3*	5.3*	6.1*	*NS*
F × L	*NS*	*NS*	*NS*	*NS*	*NS*	*NS*	*NS*	*NS*	*NS*	*NS*
R × L	*NS*	*NS*	*NS*	*NS*	*NS*	*NS*	*NS*	*NS*	*NS*	*NS*
S × L × F	*NS*	*NS*	*NS*	*NS*	*NS*	*NS*	*NS*	*NS*	*NS*	*NS*
S × L × R	*NS*	*NS*	*NS*	*NS*	*NS*	*NS*	*NS*	*NS*	*NS*	*NS*
S × F × R	*NS*	*NS*	*NS*	*NS*	*NS*	*NS*	*NS*	*NS*	*NS*	5.2*
F × R × L	*NS*	*NS*	*NS*	*NS*	*NS*	*NS*	*NS*	*NS*	*NS*	*NS*
S × F × R × L	*NS*	*NS*	*NS*	*NS*	*NS*	*NS*	*NS*	*NS*	*NS*	*NS*

(*** $P < 0.001$, ** $P < 0.01$, * $P < 0.05$, *NS* = no significance).

Average belowground biomass was 2.3-fold greater at Koeberg than at Vergaderingskop but no differences occurred in aboveground biomass between sites (Table 2.4, Fig. 2.5). Within sites, light availability affected biomass, however, significant differences were only observed between seedlings grown under the lowest (0-20%) and highest (80-100%) light availabilities (Table 2.4, Fig. 2.5). Fertilization resulted in seedlings with biomass *ca*. 2-fold greater than that of unfertilized seedlings and seedlings planted in root exclusion tubes exhibited a *ca.* 4-6-fold greater biomass than seedlings not in root exclusion tubes (Table 2.4, Fig. 2.6).

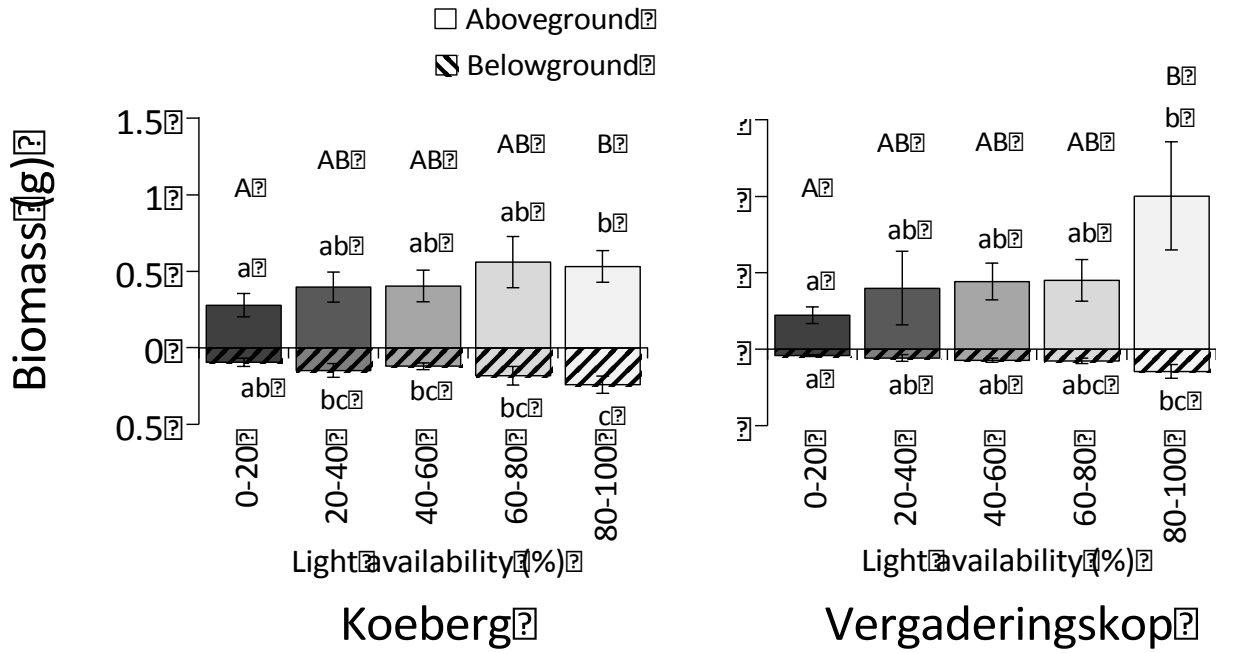

Figure 2.5. Aboveground and belowground biomass of seedlings under the range of light availabilities at Koeberg and Vergaderingskop study site. Bars represent mean ± SE. Different lower case letters indicate significant differences ($P < 0.05$) of aboveground and belowground differences between treatments, while uppercase letters indicate significant differences in the total biomass between treatments. Post hoc differences were determined by comparing contrasts of least-squares means.

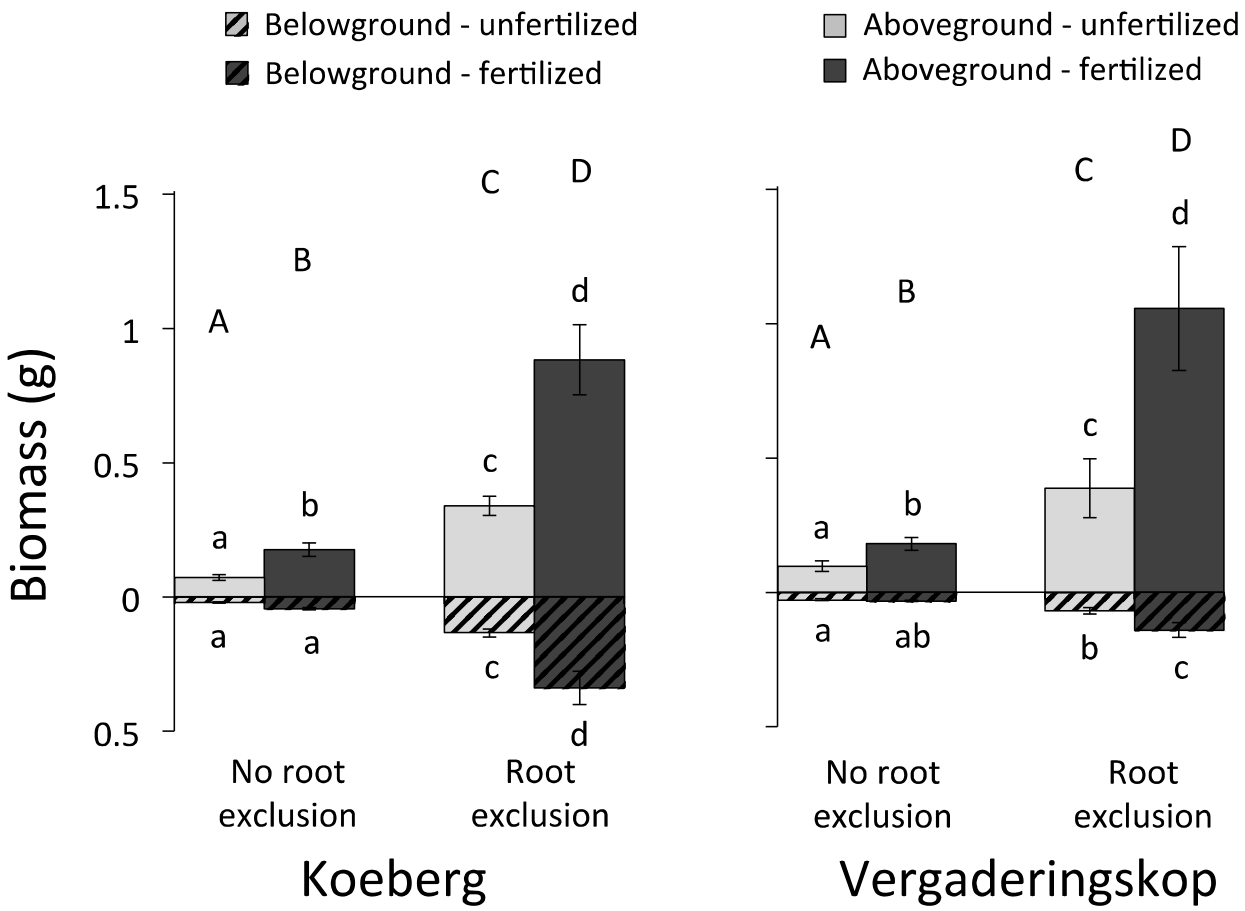

Figure 2.6. Aboveground and belowground biomass of unfertilized and fertilized *A. cyclops* seedlings without and with root exclusion tubes at Koeberg and Vergaderingskop study site. Bars represent mean ± SE. Dissimilar lowercase letters indicate significant differences ($P < 0.05$) of aboveground and belowground differences between treatments, while uppercase letters indicate significant differences in the total biomass between treatments. Post hoc differences were determined by comparing contrasts of least-squares means.

The significant differences in belowground biomass between sites resulted in significant differences in biomass allocations of seedlings. This, however, was dependent on fertilization treatment (Table 2.4). While no difference occurred in shoot:root biomass ratios in unfertilized seedlings between sites, shoot:root biomass ratios of seedling at Vergaderingskop were *ca.* 1.5-fold greater than those at Koeberg when treated with fertilizer (Fig. 2.7) . Within the Koeberg study site, shoot:root ratios were similar between seedlings regardless of treatment. At Vergaderingskop, seedlings treated with fertilizer exhibited ratios *ca*. 1.6-fold greater than unfertilized seedlings (Fig. 2.7).

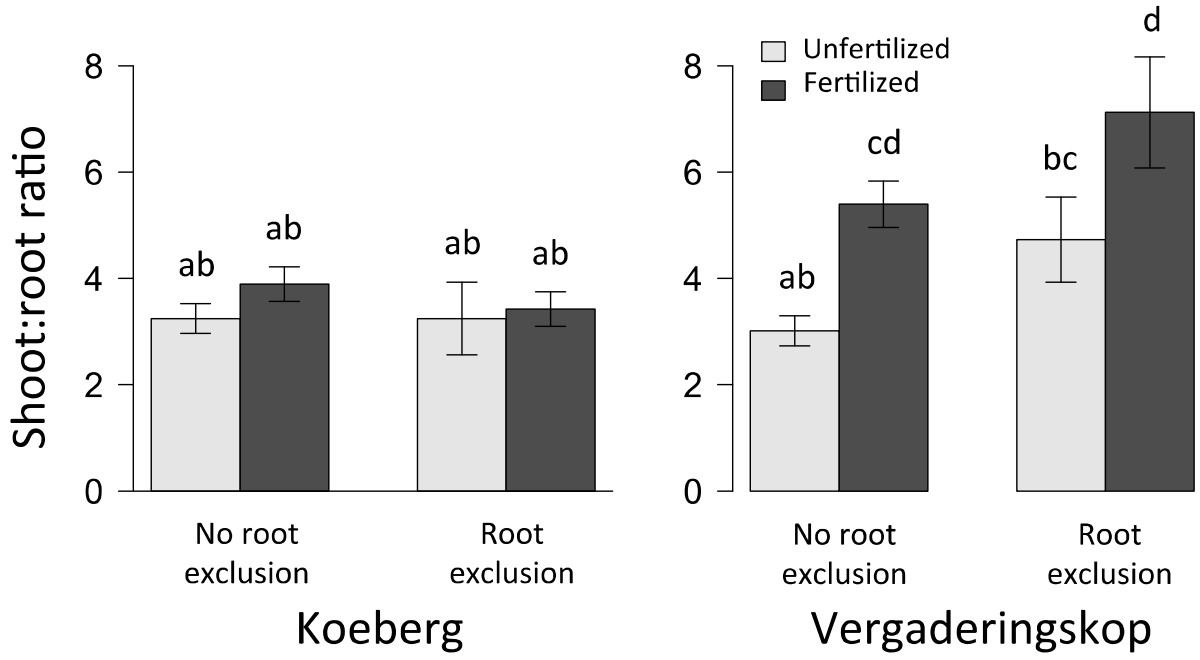

Figure 2.7. Shoot:root biomass ratios (mean ± S.E.) of unfertilized and fertilized *A. cyclops* seedlings without and with root exclusion tubes at Koeberg and Vergaderingskop study site. Bars represent the mean ± SE and dissimilar letters above bars indicate significant differences ($P < 0.05$) between treatments determined by comparing contrasts of least-squares means.

Foliar $\delta^{13}C$ values were 15% more negative, indicating greater amounts of time with stomata open, at Vergaderingskop than at Koeberg. Foliar $\delta^{13}C$ did not differ with light availability at either site (Table 2.4, Fig. 2.8). At Koeberg, unfertilized seedlings had more negative $\delta^{13}C$ than fertilized seedlings but no differences in root exclusion treatments were exhibited (Table 2.4, Fig 2.8). Whereas, at Vergaderingskop, although fertilized seedlings still exhibited more negative $\delta^{13}C$, seedlings treated with root exclusion tubes also exhibited more negative $\delta^{13}C$ than seedlings without exclusions (Table 2.4, Fig. 2.8).

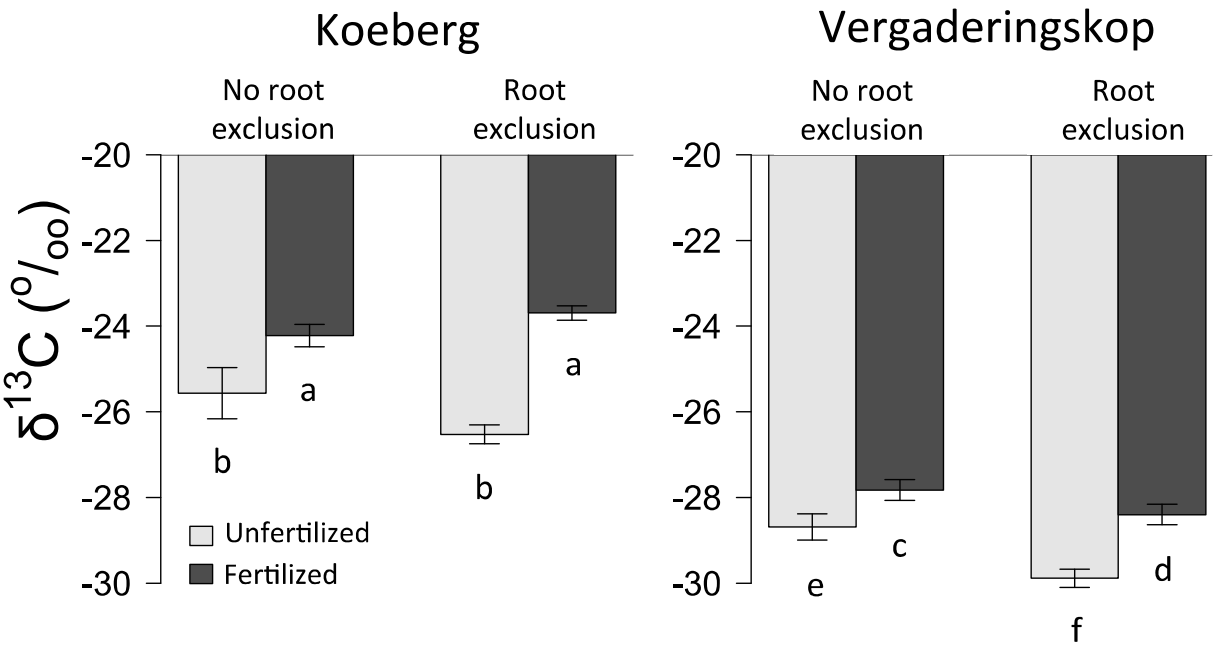

Figure 2.8. Foliar $\delta^{13}C$ of unfertilized and fertilized *A. cyclops* seedlings without and with root exclusion tubes at Koeberg and Vergaderingskop study site. Bars represent the mean ± SE. Different letters above bars indicate significant differences ($P < 0.05$) between treatments determined by comparing contrasts of least-squares means.

Average foliar N values of seedlings were greater at Vergaderingskop than at Koeberg but only for root exclusion tube seedlings (Table 2.4, Fig. 2.9). At Koeberg, unfertilized seedlings exhibited similar foliar N, while fertilized seedlings grown in root exclusion tubes exhibited foliar N *ca*. 40% greater than the other seedlings. At Vergaderingskop fertilized seedlings had foliar N *ca*. 25% greater than unfertilized seedlings, while root exclusion tubes resulted in seedlings with *ca.* 30% greater foliar N (Table 2.4, Fig. 2.9).

Nodule mass, relative to total plant biomass, was *ca.* 2-fold greater at Koeberg, than at Vergaderingskop (Table 2.4, Fig. 2.9). At both sites, the average nodule mass of fertilized seedlings was close to zero. Nodule mass of unfertilized seedlings, however, was *ca.* 2-fold greater in seedlings without root exclusion tubes (Table 2.4, Fig. 2.9), although he high variability at Koeberg resulted in statistical significance only at Vergaderingskop (Table 2.4, Fig. 2.9).

Foliar $\delta^{15}N$ values of seedlings at Koeberg were lower than seedlings at Vergaderingskop. Foliar $\delta^{15}N$ differed for fertilizer and root exclusion treatments differently between sites (Table 2.4, Fig. 2.9). At Koeberg, $\delta^{15}N$ did not differ between fertilized and unfertilized seedlings. Root exclusions resulted in higher $\delta^{15}N$ values but only in unfertilized seedlings. At Vergaderingskop, unfertilized seedlings with no root exclusion tube had 4–6-fold lower $\delta^{15}N$ than all other seedlings (Table 2.4, Fig. 2.9).

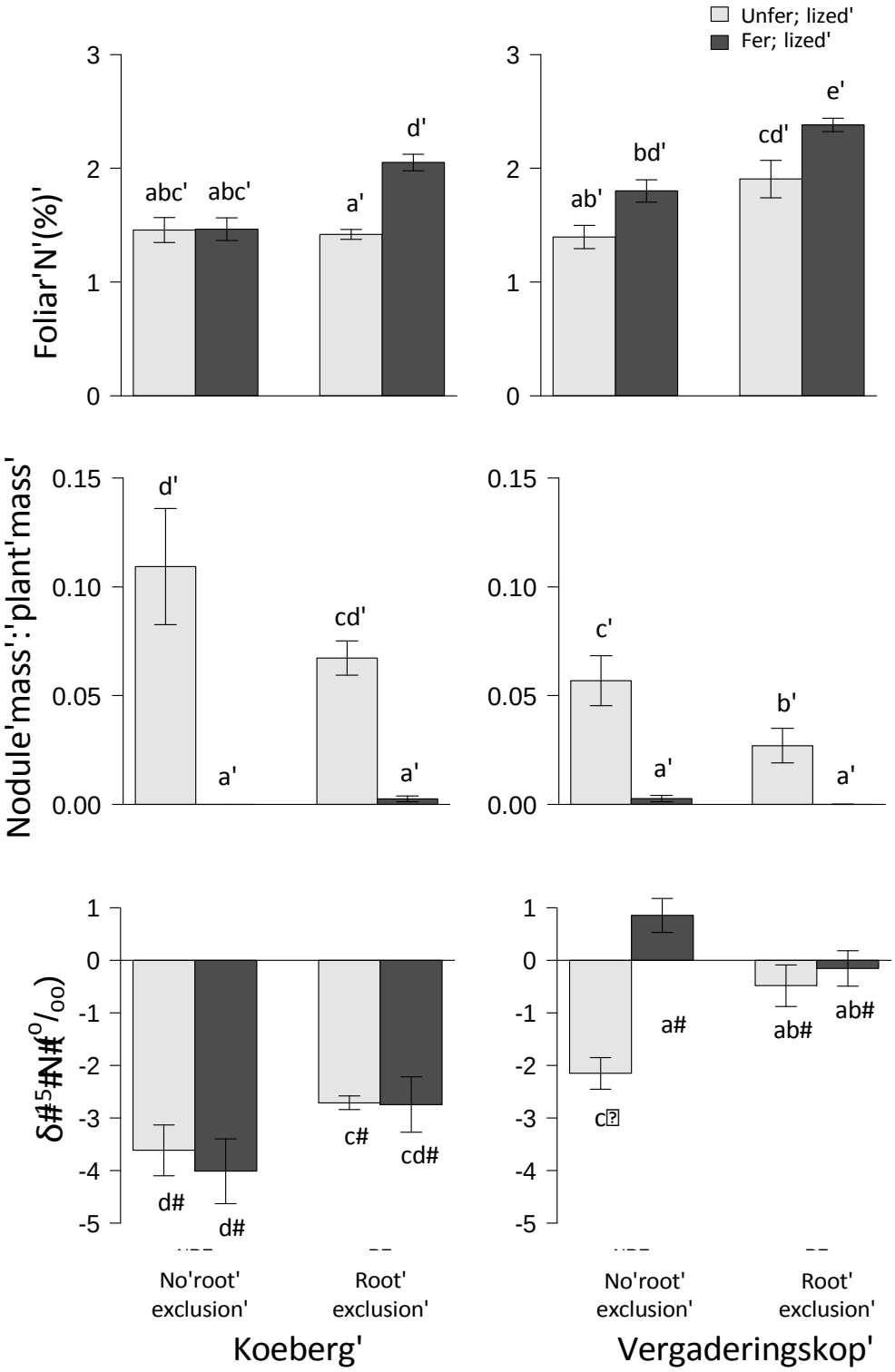

Figure 2.9. Foliar N, nodule mass relative to total plant mass and $\delta^{15}N$ of unfertilized and fertilized *A. cyclops* seedlings without and with root exclusion tubes at each site. Bars represent the mean ± SE and dissimilar letters above the bars indicate significant differences ($P < 0.05$) between treatments determined by comparing contrasts of least-squares means.

2.5 Discussion

Survival of control seedlings varied from 20% at Koeberg to 50% at Vergaderingskop. Competition from native vegetation played an important role in limiting survival of these seedlings since when competition was removed survival increased to 60% and 70% respectively. Considering the copious seed production and continuous spread of *A. cyclops* seeds into native vegetation patches, constraints of competition on seedling survival are unlikely to completely limit invasion. The constraints placed on seedling performance through native vegetation competition, however, may play an important role in limiting the rate and extent of invasions spread thereby constraining rather than preventing invasion and thus may be an important bottleneck to invasion success.

Seedling survival was equally likely across all light availabilities, however, as hypothesized light availability differentially constrained seedling performance. Under the lowest light availability biomass accumulation, both aboveground and belowground, were reduced despite light availability having no affect on seedling height. Similar results were found for seedlings of the closely related *A. longifolia*, which exhibited a 3-fold decrease in biomass but not height under reduced light conditions (Peperkorn *et al*. 2005). These results indicate that aboveground competition may not be the main determinant limiting seedling recruitment but that growth may be slowed under high levels of aboveground competition for light. Results suggest that seedlings are able to tolerate a wide range of light availabilities. Heteroblasty has been proposed as an exaptation (*sensu* Gould and Vrba 1982) to light availability in phyllodinous *Acacia* species with bipinnate leaflets suggested to be an advantage for early growth and shade tolerance (reviewed in Pasquet-kok *et al*. 2010). While the transitions from bipinnate leaflets to phyllodes were not recorded in this

study, heteroblasty in these seedlings may have enhanced seedling tolerance to the range of light availabilities.

Belowground competition is potentially an important determinant of seedling survival since when reduced, *A. cyclops* seedlings were twice as likely to survive. Belowground competition for water is likely to have been a factor that contributed to seedling mortality since survival differences were observed between seedlings without and with root exclusion tubes even when nutrient constraints were ameliorated through fertilization. This would be congruent with the notion of Coomes and Grubb (2000), who suggest that water availability places a greater constraint on seedling establishment than nutrient availability since a lack of water tends to result in seedling mortality whereas a lack of nutrients is likely to constrain growth.

Of seedlings that did survive, belowground competition also constrained seedling performance as was evident by the 50% increase in height and 5-fold greater biomass of seedlings when protected from belowground competition. These differences are likely to be in part due to competition for water resources since when nutrient constraints were ameliorated through fertilizer additions, seedlings protected from belowground competition still exhibited greater biomass and height than seedlings subject to belowground competition. Constraints of belowground competition are, however also associated with competition for soil nutrients, since fertilizer additions resulted in a 20% height increase and a doubling in biomass of seedlings. The influence of nutrient availability on the performance of invasive Australian *Acacia* seedlings has been shown in other species including *A. saligna* which exhibited increased mass in response to increasing

nutrient availability (Witkowski 1991) and *A. longifolia* which had greater heights and biomass under higher nutrient availabilities (Peperkorn *et al*. 2005).

Seedlings exposed to root competition exhibited similar foliar N concentrations regardless of whether they were fertilized or not. The significantly greater biomass of nodules on unfertilized seedlings may indicate that under low nutrient conditions, *A. cyclops* seedlings rely, in part, on N_2-fixation to acquire N. This would be congruent with several studies that have indicated N_2-fixation to be an important trait contributing to the success of invasive Australian *Acacias* (reviewed in Morris *et al*. 2011) especially when growing in nutrient poor environments (Rodríguez-Echeverría *et al*. 2009).

Foliar $\delta^{15}N$ values close to zero or below (due to fragmentation during fixation) are generally indicative of the occurrence of N_2 fixation (Lajtha and Marshall 1994) and previously reported foliar $\delta^{15}N$ values of N_2-fixing *A. cyclops* seedlings ranged between 0 to -2 ‰ (Stock *et al.* 1995). The strongly negative $\delta^{15}N$ values of seedlings in this study (-2 to -4 ‰) are thus slightly more negative than should be expected for N_2-fixing plants. Variations in foliar $\delta^{15}N$ values can be associated with differences in rooting depth, utilization of different N pools or due to variations in symbiotic mycorrhizal associations (Spriggs *et al*. 2003). However, since all *A. cyclops* seedlings are likely to be accessing similar pools of resources and the same functional guild of mychorrizal associations it is probable that differences in the negative $\delta^{15}N$ values correlate with differences in N_2-fixation activity. This would be congruent with foliar $\delta^{15}N$ results of Vergaderingskop seedlings that were exposed to root competition in which unfertilized seedlings had *ca*. 3-fold lower $\delta^{15}N$ than fertilized seedlings. At Koeberg, all seedlings exhibited negative foliar $\delta^{15}N$ (between ca. -2 to -4 ‰). Considering the significantly lower total soil N

concentrations at Koeberg in comparison to Vergaderingskop even when fertilized this may correlate with the suggested N_2-fixation activity exhibited in all seedlings at the lower fertility site.

Large differences were also exhibited in $\delta^{13}C$ between sites and between fertilized and unfertilized seedlings. Differences in $\delta^{13}C$ can be used as an indication of differences in the CO_2 concentration in the intercellular spaces of leaves (C_i) due to the discrimination of ^{13}C by RuBP, which in turn can be used as an indicator of the relative dynamics of stomatal regulation (Farquhar *et al.* 1982). This has often led to $\delta^{13}C$ being used as a correlate of WUE (Farquhar *et al.* 1982). However, $\delta^{13}C$ and WUE are not always positively correlated (e.g. Akhter *et al.* 2005). Thus it is recognized that differences in $\delta^{13}C$ values can also be indicative of other processes that may regulate stomatal regulation other than through assimilation of CO_2. Sparks and Ehleringer (1997) propose that lower nutrient conditions correspond to decreased photosynthetic activity yielding increased intercellular leaf CO_2 conditions and hence more $\delta^{13}C$ discrimination. Alternatively, suggestions that under limited nutrient conditions, increased stomatal conductance may be associated with transpiration-driven mass flow as a nutrient acquisition strategy has been proposed (Cramer *et al.* 2009). Therefore it is important to consider that $\delta^{13}C$ values integrated over the lifetime of a plant may arise due to multiple processes regulation stomatal conductance. The consistently more negative $\delta^{13}C$ of unfertilized plants in my results may support suggestions that low nutrient conditions stimulate transpiration driven mass flow, which would result in increased stomatal conductance and decreased $\delta^{13}C$. Additionally, the lower $\delta^{13}C$ value at Vergaderingskop in comparison to Koeberg may indicate lower water

limitations at this site since the rainfall at Vergaderingskop is less strictly winter biased and tends to fall more regularly throughout the year.

In conclusion, results suggest that belowground competition is important in constraining invasive tree seedling survival in Mediterranean type ecosystems. If seedlings are able to survive, competition from native vegetation constrains growth and biomass accumulation potentially reducing the rate of maturation and hence invasive spread of these species. Belowground competition appears to place the greatest constraints on invasive tree seedlings and thus root gaps, which may not always be correlated with canopy gaps (Esler *et al.* 2002), may promote invasive tree seedling establishment. These results provide evidence for the importance of maintaining intact undisturbed native ecosystems that are able to constrain invasion establishment and slow invasion spread allowing greater windows of opportunity for management procedures. Additionally, understanding the mechanisms that constrain recruitment of invasive tree seedlings into intact native vegetation may be useful in predicting invasion risks of different plant communities, identifying important features for ecosystem restoration and informing invasive plant management strategies.

CHAPTER 3
THE ROLE OF GENERALIST NATIVE HERBIVORES IN CONSTRAINING INVASIONS OF ALIEN TREES: *RHABDOMYS PUMILIO* LIMITS *ACACIA CYCLOPS* ESTABLISHMENT

3.1. Abstract

Regulation of non-native invasive species by native generalist herbivores is a factor of biotic resistance to alien plant invasions. Despite the recognition that seedling herbivory is the most frequent cause of mortality in trees, few examples of native herbivory on invasive tree seedlings exist. Australian *Acacias* have become widely invasive in the Cape Floristic Region of South Africa, however, little is known on the role of herbivory in constraining seedling establishment of invasive tree seedlings. I thus hypothesized that seedling herbivory by native herbivores limits recruitment of *Acacia cyclops* in undisturbed native ecosystems. To test this I transplanted *A. cyclops* seedlings into native CFR coastal vegetation under three different herbivore exclusion treatments. Seedlings were monitored for eight days after planting. Rapid herbivory of 60-100% of *A. cyclops* seedlings occurred, largely attributable to the abundant three-striped mouse, *Rhabdomys pumilio*. Seedling herbivory was not dependent on rodent density (200-300 mice ha^{-1}), which was high across all sites (3-fold greater than other vegetation types). Instead, seedling herbivory decreased under low vegetation cover with open sandy patches. This indicated that herbivory is correlated with vegetation structure, which determines the behaviour of *R. pumilio* since the diurnal rodent is known to avoid foraging in open areas. Result provides evidence to suggest that generalist herbivores constrain invasive tree recruitment in undisturbed native ecosystems. Such information may be useful in predicting resistance of different ecosystems to alien tree invasions and for identifying vegetation characteristics that may enhance resistance to reinvasion during restoration efforts.

3.2. Introduction

The release of an introduced plant species from its co-evolved specialist enemies, has long been acknowledged as an important factor contributing to invasion success (Darwin 1859, Elton 1958, Crawley 1987, Keane and Crawley 2002). Recognition that these introduced plants are likely still subject to regulation by generalist enemies in the new environment is a key factor underpinning the theory of biotic resistance (Elton 1958). Herbivory, is recognized as a key factor of biotic resistance and has been shown to reduce seed abundance, establishment and growth of introduced alien invasive plant species (Levine *et al.* 2004). Despite the extensive body of literature (reviewed in Levine *et al.* 2004, Maron and Vilà 2001, Parker *et al.* 2006), evidence for the role of indigenous herbivores to limit invasive trees is sparse.

For tree species, earliest life stages are often most sensitive to herbivore-induced mortality. Several examples exist demonstrating the effect indigenous herbivores can have on reducing seed loads of invasive tree species (Holmes 1990, Mokotjomela and Hoffmann 2013, Nuñez *et al*. 2008) but whether this results in invasion constraint is largely unknown and is likely to depend on the magnitude and extent of seed input into the system (D'Antonio *et al*. 2001). Herbivory of seedlings, however, is likely to have a direct impact on invasion establishment. Despite recognition that herbivory is the most frequent cause of native tree seedling mortality (Moles and Westoby 2002), few examples of herbivory by indigenous herbivores on invasive tree seedlings exist.

Australian *Acacias* are widely invasive in the Cape Floristic Region (CFR) of South Africa (Henderson 2007, Richardson and Rejmánek 2011). In their native range, herbivory by invertebrate specialists is prevalent with seeds, stems, roots and foliage targeted by

several specific species of beetles, bugs and Lepidoptera (Lawrence and Britton 1994, Hadlington and Johnson 1996, Zborowski and Storey 1995). Herbivory by generalists is less common although seedlings are sometimes eaten by indigenous vertebrates such as wallabies and possibly also wombats, along with introduced livestock of sheep, goats and rabbits (Hadlington and Johnson 1996). Native bird species feed on seeds of several *Acacia* species, which are often adapted for bird dispersal having brightly colored nutrient rich arils attached to seeds.

Acacia cyclops A. Cunn. Ex. Don (Fabaceae) is one of the most prevalent invasive Australian *Acacias* in the CFR (Henderson 2007) supported by copious seed production in introduced ranges (1000-3000 seed rain m^{-2} canopy; reviewed in (Gibson *et al*. 2011). While invasions are commonly associated with areas disturbed by fire or land use practices, the species is also able to colonize intact undisturbed native vegetation (Stirton 1980, Richardson *et al*. 1997). Spread into native vegetation is often attributed to native bird species that have been shown to defecate or regurgitate ingested seeds large distances away from the invasion source (Fraser 1990, Glyphis *et al.* 1981, Milton and Hall 1981, Underhill and Hofmeyr 2007).

In the introduced range in the CFR, herbivory of seeds by native fauna has been recorded. The generalist three striped mouse, *Rhabdomys pumilio*, has been shown to consume between *ca*. 80-90% of *A. cyclops* seeds off the ground (Holmes 1990b, Mokotjomela and Hoffmann 2013). In addition, ants in the genera *Anoplolepis* and *Pheidole* rapidly remove and bury *A. cyclops* seeds from below the canopy into their belowground nests resulting in local scale dispersal, as well as contributing to soil seed bank (Holmes 1990). However, the fate of seedlings to herbivory, is as yet undocumented for any of the

invasive Australian *Acacia* species. I thus hypothesized that seedling herbivory by indigenous herbivores limits recruitment of *A. cyclops* in undisturbed native ecosystems. To test this I transplanted *A. cyclops* seedlings into native CFR coastal vegetation and monitored survival. Identities of herbivores and predictors of herbivory were also assessed.

3.3. Methods

3.3.1. Study sites

I established study sites at Koeberg Nature Reserve (33.65287 S, 18.43725 E) and Vergaderingskop Nature Conservancy (34.55466 S, 19.37298 E) in the CFR region of the Western Cape of South Africa. The sites were chosen based on the criteria of being areas of conserved native vegetation in which stands of *A. cyclops* invasions have established. Sites were located in the coastal vegetation communities, commonly referred to as "strandveld", associated with the well-drained marine-derived aeolian sands. Vegetation consists of broad-leaved, sclerophyllous shrubs often with fleshy fruit such as the genera *Searsia*, *Euclea*, *Olea*, *Cassine*, *Cussonia*, *Zygophyllum* and *Maytenus*, which interspersed with succulents, bulbs and grasses and is generally low in stature with vegetation heights of 1-2 m (Mucina and Rutherford 2006). Coastal habitats in the CFR have been progressively invaded by *A. cyclops*, which was originally introduced from southwestern Australia into the CFR in the 19th century for the purpose of dune stabilization (Poynton, 2009).

3.3.2. Seedling herbivory

Three experimental replicates were established in the native strandveld vegetation at each study site, a minimum of 1 km apart. Experiments were conducted in the dry summer season (February 2013) and the wet winter season (July/August 2013). *Acacia cyclops* seeds, collected from Vergaderingskop in December 2012, were scarified with sandpaper and grown in trays of sand/potting soil mix in the University of Cape Town Botany greenhouse. Upon germination approximately 10 days after sowing, 60 seedlings were planted in each experimental replicate in a 5 x 12 array spaced *ca.* 5 m apart for a total of 180 seedlings at each site.

Seedlings were assigned to three different treatments that altered the exposure of herbivory to different herbivore guilds. Ten randomly selected seedlings in each replicate were protected from mammal herbivory, but open to invertebrate herbivory by mesh cages 20 cm in diameter and 30 cm in height (mesh size 2.5 cm) (designated as "Full cage"). A further ten randomly selected seedlings were protected from large vertebrate herbivory, but open to small vertebrate herbivory with a wire mesh cages as above, but with a *ca.* 5 x 5 cm opening established at ground level (designated as "Partial cage"). The remaining 40 seedlings were left open to all herbivory (designated as "No cage"). Seedling locations were marked with small flags constructed of steel pegs and tape. Seedlings were monitored every second day for eight days for herbivory, which was defined as complete removal of seedlings (usually accompanied by digging) or removal of photosynthetic material (cotyledons and bipinnate leaflets; Fig. 3.1).

Figure 3.1. Image illustrating definition of herbivory as removal of photosynthetic plant parts of *A. cyclops* seedlings before (left) and after (right) herbivory.

3.3.3. Small mammal community assessment

The same replicate sites were used for small mammal population assessments. In each, 25 Sherman live-traps (5.2 x 6.4 x 16.5 cm) were placed in a 5 x 5 array *ca*. 10 m apart. Traps were covered with polyurethane covers to buffer extreme temperatures, cotton wool was provided as bedding for thermoregulation and apple pieces were provided as a hydration source. Traps were baited with a peanut butter/oats combination mixed to a consistency where bait balls (1x1 cm) could be formed. Chicken feed seed mix was placed at the entrance to taps once placed on the ground. Traps were baited and locked open for two nights to acclimatize small mammals to the traps. Thereafter, traps were baited with the same bait mix in the afternoons between 3:00 – 5:00 pm for four consecutive nights equating to 300 total trap nights at each site for each of the summer and winter season (2400 trap nights in total). Traps were checked each morning between 7:00–10:00 am and left closed for the remainder of the day. Trapped individuals were identified and marked by cutting a small section of fur off the dorsal hind region before being released. Small

mammal density was estimated using the Schumacher and Eschmeyer Method (Krebs 1999), for closed populations on species with sufficient captures. All methods described herein were approved by the University of Colorado Institutional Animal Care and Use Committee (Protocol 1307.01) as well as the University of Cape Town Animal Ethics Committee (Protocol 2012/V48/JM).

3.3.4. Vegetation structure and community

Vegetation variables were measured in each replicate to determine correlations to herbivory patterns. Several vegetation structure variables were measured in each replicate on three 20 m transects spaced 20 m apart (Fig. 3.2) including: percent ground cover of soil, herbaceous vegetation and litter, which were estimated in a 1 x 1 m quadrat every 5 m along each transect; maximum vegetation height within a 2m radius every 5m along each transect measured with a metered ranging pole; canopy cover which was assessed every 5 m along each transect using a spherical densitometer that was held at breast height and the number of squares covered by canopy cover in the reflective curved mirror counted (Lemmon 1956). Vertical foliage density (m^2 m^{-3}) was estimated using the cover board methods of MacArthur and MacArthur (1961), and modified by Ralph (1985). The distance away from the transect line at which the board was 50% obscured by foliage, in the height classes of 0-0.5 m, 0.5-1 m, 1-1.5 m and 1.5-2 m, was measured to the left and right every 5 m along the central transect (Transect 2, Fig. 3.2). The average distance (D) for each height class was calculated and the corresponding foliage density (k) was estimated using the equation $k = 0.69315/D$ according to Ralph (1985).

Vegetation community variables measured included: Herbaceous species count, which was estimated using the line intercept method on the three transect lines (Fig 3.2), where every 1 m a pinflag was placed and the herbaceous vegetation under the flag was identified. If no vegetation occurred within a 0.5 m radius from the flag it was recorded as empty. The number of native woody species was determined by identifying all native woody species (stem diameter > 3 cm) in three 25 x 2 m wide belt transects spaced 20 m apart (Fig 3.2).

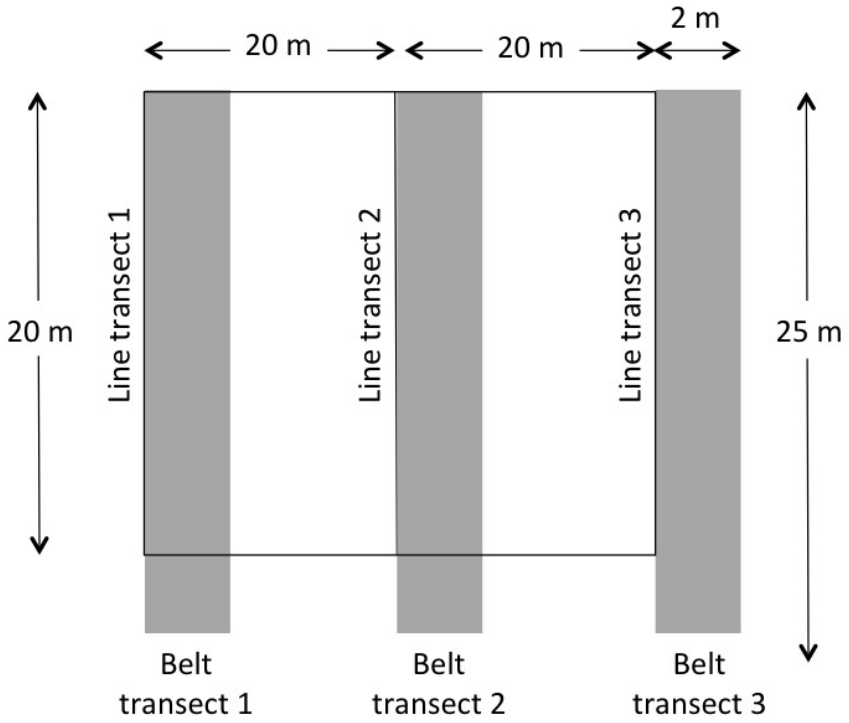

Figure 3.2. Layout of vegetation sampling transects in each experimental replicate. Line transects were used for sampling ground cover, canopy cover, vegetation height, vegetation density (transect 2 only) and herbaceous species richness. Woody species richness was determined in belt transects.

3.3.5. Statistical analysis

I used mixed effects models implemented in R (R Core Team 2012), to analyze *A. cyclops* seedling herbivory and mammal population density. Mixed effects models allow for a combination of fixed effects, which influence the mean of the population, and random effects, which influence the variance around the mean. Variables associated with entire populations or repeatable experimental treatments are specified as fixed effects, while random effects are specified according to experimental units drawn at random from a population that govern the variance within the response variable (Pinheiro and Bates 2000). This makes mixed effects models suitable for data that may be correlated by space (or time) due to sampling replications (Crawley 2007, Zuuer *et al.* 2009). Thus, potential autocorrelation between block replicates at each site could be taken into account by specifying the different sites as a random effect in mixed effects models. Mixed effects models are also well suited for unbalanced designs which were present for the seedling herbivory experiment that had replicates of 10 caged seedlings, 10 partially caged seedlings and 40 uncaged seedlings at each site.

I used generalized linear mixed effects model to analyze the binomial response variable of *A. cyclops* seedling herbivory (dead=0, alive=1) with the *glmer* function in the *lme4* package for R (Bates *et al.* 2013). I specified the full model to include fixed effects as a three-way interaction between site (Koeberg and Vergaderingskop), season (summer and winter), and cage treatment (full cage, partial cage and no cage) and random effects as replicate nested within site. For small mammal species with sufficient captures, population density was analyzed using a linear mixed effects model with the function *lmer* also in the

lme4 package. I specified the fixed effects as a two-way interaction between site and season and the random effects as replicate nested within site.

Best fit models were obtained by sequential deletion of fixed effects variables as described in (Buckley *et al*. 2003). Significance of variables retained in the final best-fit model were determined using the Wald $\chi 2$ statistic derived using the *Anova* function in the *AER* package (Kleiber and Zeileis 2008). Post-hoc differences were determined by comparing contrasts of least-squares means using the *lsmeans* function in the package *lsmeans* (Lenth 2014). Plots of fitted and observed values and residuals were examined to ensure deviations from homoscedasticity and normality did not occur as described by Pinheiro and Bates (2000).

To test for correlations between *A. cyclops* seedling herbivory and measured environmental correlates, simple linear regressions were performed in R. Bonferonni p-values were used to assess significance of correlations, due to the large number of comparisons, and were derived using the *p.adjust* function in the inbuilt *stats* package in R.

3.4. Results

3.4.1. Seedling herbivory

Fully caged seedlings did not experience any herbivory at either site. Herbivory of caged seedlings and uncaged seedlings were similar within sites but between sites, eight days after planting, Vergaderingskop had higher herbivory (*ca.* 100%) than Koeberg (*ca.* 70%) (Table 3.1, Fig 3.3). Furthermore, herbivory was *ca.* 20% greater in summer than winter at Koeberg but was equally high (*ca.* 100%) in both seasons at Vergaderingskop (Table 3.1, Fig 3.3). Herbivory of uncaged seedlings, was between 60-80% at Koeberg,

while at Vergaderingskop 98-100% of seedlings experienced herbivory. At Vergaderingskop *ca*. 100% of the herbivory occurred within two days of planting whereas at Koeberg *ca.* 90% of the summer herbivory and only *ca.* 50% of the winter herbivory had occurred after two days (Fig. 3.3).

Table 3.1. Wald χ2 test statistics and *P*-values for parameters of best-fit models explaining herbivory induced mortality (a) and *Rhabdomys pumilio* density (b) as a function of site, season and where applicable cage treatment.

Fixed effect model parameters	(a) Herbivory mortality		(b) *R. pumilio* density	
	χ2	*P*	χ2	*P*
Site	27.2	< 0.001	*NS*	*NS*
Season	4.7	0.03	5.6	0.02
Treatment	486.2	< 0.001	*NS*	*NS*
Site × treatment	15.1	< 0.001	*NS*	*NS*
Site × season	4.1	0.043	*NS*	*NS*
Season ×treatment	*NS*	*NS*	*NS*	*NS*
Site × season × treatment	*NS*	*NS*	*NS*	*NS*

Table 3.2. Trap statistics and abundances of small mammal species captured at each site, both the summer and the winter season.

	Koeberg		Vergaderingskop		TOTAL
	Summer	Winter	Summer	Winter	
Gerbillurus paeba (hairy-footed gerbil)	9	19	0	0	28
Mus *minutoides* (pygmy mouse)	6	3	0	0	9
Otomys irroratus (vlei rat)	0	1	2	1	4
Rhabdomys pumilio (striped mouse)	84	118	91	118	411
Steatomys krebii (Kreb's fat mouse)	1	0	0	0	1
Total individuals	100	141	93	119	453
Total captures	203	246	216	258	923
Trap success (%)	78	102	85	104	92

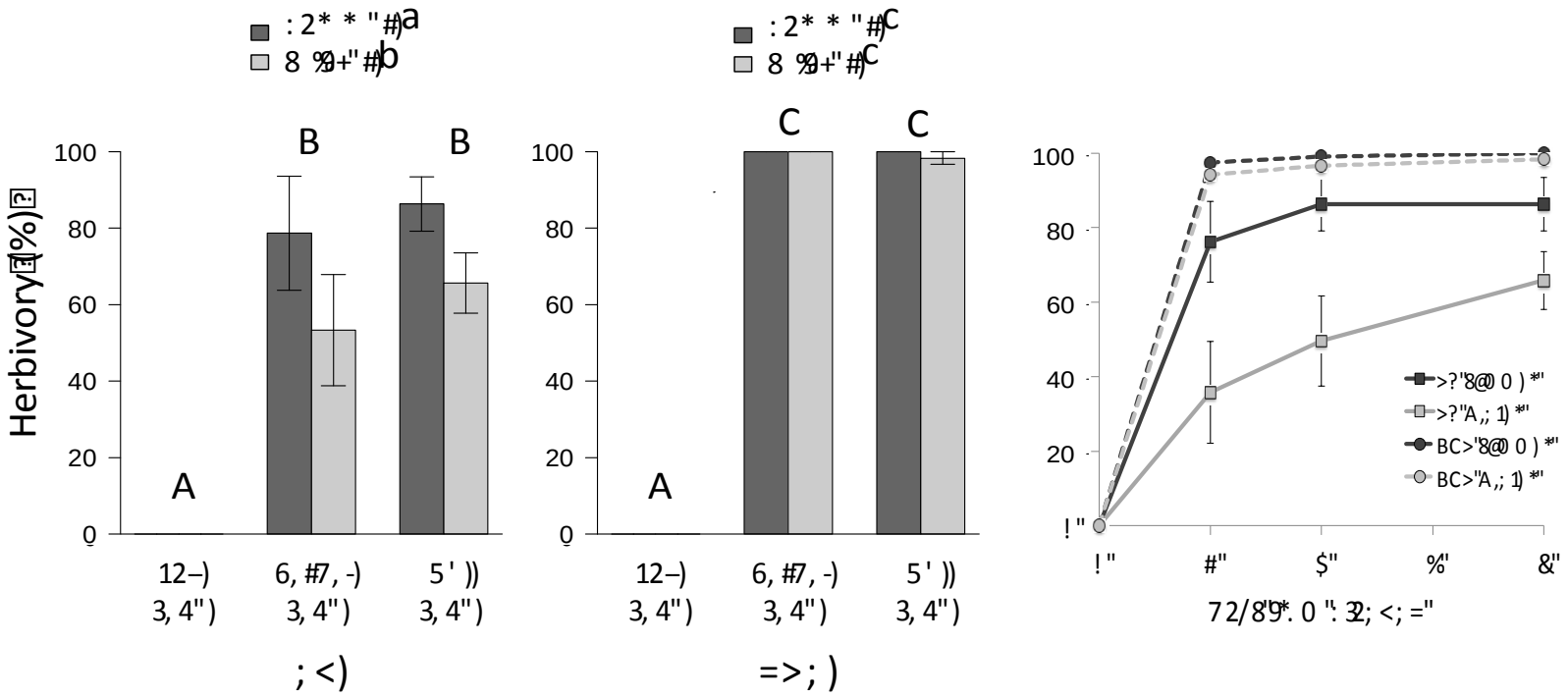

Figure 3.3. Herbivory (mean + SE) of *A. cyclops* seedlings with full cage (open to invertebrates), partial cage (open to invertebrates and small mammals) and no cage (open to all herbivory) in summer and winter at Koeberg (KB) and Vergaderingskop (VGK). Wald $\chi 2$ statistics for differences between treatments are provided in Table 2.1. Significance of site × season interactions are indicated with lowercase lettering, while significance of site × treatment interactions are indicated with uppercase lettering as determined from comparison of least square means. Cumulative herbivory mortality over time since planted is also provided for the *A. cyclops* seedlings exposed to all herbivory (no cage) indicative of native herbivory.

3.4.2. Herbivore identity

Herbivory of *A. cyclops* seedlings was not attributable to invertebrate herbivores since seedlings in full cage seedlings did not experience any herbivory (Fig. 3.3). Instead, similar herbivory levels occurred between partial cage and no cage seedlings (Table 3.1, Fig. 3.3), indicating that herbivory of the no cage seedlings was likely attributable to rodents, which were the only herbivore guild able to access seedlings in the partial cages.

Five rodent species at Koeberg and only two at Vergaderingskop were captured over a four night sampling period in winter and summer (Table 3.2). The striped mouse, *Rhabdomys pumilio*, dominated capture data comprising 84% and 99% of individuals captured at Koeberg and Vergaderingskop respectively (Table 3.2). Estimated population densities of *R. pumilio*, determined from mark recapture estimates did not differ between sites, but were higher in winter than in summer (Table 3.1, Fig 3.4.). Despite sufficient trap numbers, trap success rates of greater than 100% occurred due to captures of multiple individuals in a single trap. Trap success ranged from *ca.* 80-110% (Table 3.2).

3.4.3 Environmental predictors of herbivory

Herbivory of *A. cyclops* seedlings did not depend on small mammal abundance or vegetation community composition, but was correlated with ground cover and vegetation structure variables (Table 3.3). Increased soil ground cover (R^2 = 0.59, P = 0.02) and decreased litter ground cover (R^2 = 0.68, P = 0.008) resulted in decreased herbivory of *A. cyclops*. Herbivory of *A. cyclops* seedlings increased with increasing vegetation height (R^2 = 0.63, P = 0.02) as well as with biomass density of vegetation between 0.5-1 m (R^2 = 0.52, P = 0.04) and 1.0-1.5 m (R^2 = 0.63, P = 0.02) (Table 2.3).

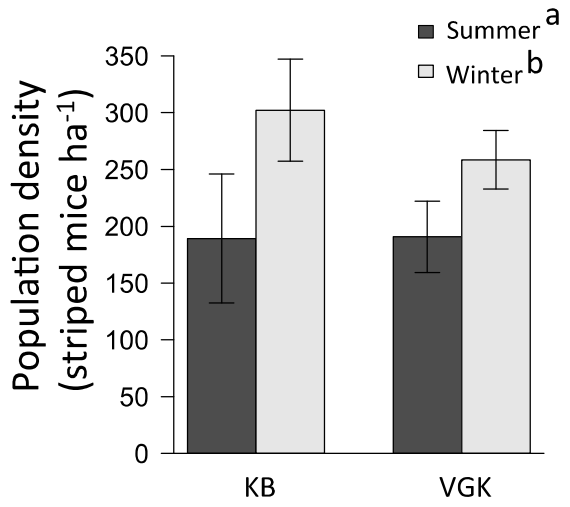

Figure 3.4. Population density estimates (mean + SE) of striped mice (*Rhabdomys pumilio*) in the summer and winter season at Koeberg (KB) and Vergaderingskop (VGK) sites. Populations did not differ by site but were higher in the winter season as indicated by lowercase lettering (Table 3.1).

Table 3.3. Results of linear regression analyses testing the dependent variable of herbivory mortality of *A. cyclops* seedlings against independent variables categorized into groups relating to small mammal abundance (a), ground cover (b), vegetation structure (c), and vegetation community structure (d). P-values are Bonferonni corrected. Variables with significant correlation to seedling herbivory are indicated with bold R^2 and *P* values.

Variable group	Independent variable	*d.f.*	*slope*	*y-intercept*	*adj. R^2*	*P*
(a) Small mammal abundance	*Rhabdomys pumilio* density	10	-0.8	118.2	0.21	1
(b) Ground cover	Soil cover (%)	10	-0.5	102.8	**0.59**	**0.02**
	Herbaceous cover (%)	10	0.9	68.6	0.34	0.3
	Litter cover (%)	10	0.9	45.3	**0.68**	**0.008**
(c) Vegetation structure	Vegetation height (m)	10	37.0	29.9	**0.63**	**0.02**
	Canopy cover (%)	10	7.4	76.5	0.13	1
	Vertical biomass density: 0-0.5 (m^2 m^{-3})	10	25.1	67.1	0.32	0.36
	Vertical biomass density: 0.5-1 (m^2 m^{-3})	10	98.3	47.1	**0.52**	**0.04**
	Vertical biomass density: 1-1.5 (m^2 m^{-3})	10	110.0	65.2	**0.63**	**0.02**
	Vertical biomass density: 1.5-2 (m^2 m^{-3})	10	197.8	66.8	0.37	0.25
(d) Vegetation community	Herbaceous species richness	10	1.3	67.7	0.35	0.31
	Woody species richness	10	2.1	70.8	0.24	0.73

3.5 Discussion

This study shows that generalist herbivores can reduce invasive tree recruitment in undisturbed native ecosystems. I found 65-100% *A. cyclops* seedlings were consumed in undisturbed native strandveld study sites within eight days of planting. The high levels of herbivory, which entailed removal of all foliar material or entire seedlings, is indicative of vertebrate herbivory attributed to their larger size, greater mobility, broader diets and ability to consume whole plants or plant parts (Hulme 1994, Parker *et al.* 2006). Cage treatments suggest that herbivory was attributable to small rodents, likely the abundant generalist rodent, *R. pumilio*.

Seedling herbivory was not correlated with *R. pumilio* density (200-300 mice ha^{-1}) despite the rodent density being consistently high across all sites. *Rhabdomys pumilio* densities have been reported at between 35-93 mice ha^{-1} in a South African grassland (Brooks 1974), at *ca.* 150 mice ha^{-1} in a South African arid ecosystem (Schradin and Pillay 2006), while densities between 30-300 mice ha^{-1} have been found in other strandveld regions (David and Jarvis 1985). Thus densities captured in study sites were in the upper range of recorded densities. Instead, seedling herbivory decreased under low vegetation covers in open sandy patches, indicating that herbivory may instead depend on the behaviour of *R. pumilio*. This would consistent with results from other regions that show *R. pumilio* abundance to decrease in low vegetation cover (Monadjem 1997, Els and Kerley 1996).

Behavioral patterns of *R. pumilio* could explain the site level differences of *A. cyclops* seedling herbivory between Vergaderingskop and Koeberg. Herbivory was greater at Vergaderingskop despite similar densities of *R. pumilio*, which may be attributable to

differences in vegetation structure since Vergaderingskop has greater vegetation height, vegetation density (1-1.5m height class) and fewer bare sand patches (Table A1). The overall high prevalence of seedling herbivory associated with *R. pumilio* at two sites *ca.* 400 km apart however, may indicate that this is a feature that is generalized to native strandveld vegetation. Whether similar herbivory patterns exist for other invasive tree species widely prevalent within the CFR would be useful in providing further context to the role of biotic resistance to alien tree invasion through seedling herbivory by generalist herbivores.

In conclusion, results indicate that generalist rodent species may limit recruitment of invasive tree species in native ecosystems. Understanding the drivers of seedling herbivory may be useful in informing management decisions on removal strategies (e.g. avoiding removal of native understory species) and restoration targets in already invaded areas.

CHAPTER 4
ECOPHYSIOLOGICAL TRAITS ASSOCIATED WITH THE COMPETATIVE ABILITY OF INVASIVE AUSTRALIAN ACACIAS

Morris, T.L., Esler, K.J., Barger, N.N., Jacobs, S.M., and Cramer, M.D. (2011). Ecophysiological traits associated with the competitive ability of invasive Australian acacias. *Diversity and Distributions* 17(5): 898–910.

4.1 Abstract

I explored morphological and ecophysiological traits that enable invasive Australian acacias to compete with native species for resources (light, water and nutrients) necessary to support the substantial growth associated with successful invasions. Invasive Australian acacias grow large and seed prolifically in invaded regions. The greater capacity for vegetative growth is underpinned by their ability to acquire and efficiently use resources in non-native habitats. Key biological traits that enhance acquisition include 1) rapid and substantial allocation to root mass (up to 6-fold more than co-occurring native species) directed towards deep roots (at least 50% longer than those of natives) and to extensive shallow root networks; 2) heteroblasty, in most species, conferring high relative growth rates as bipinnate seedlings but long-lived, nutrient-conserving phyllodes as adults and 3) strong N_2-fixation abilities. The ecophysiological traits that govern the competitive interaction of invasive Australian acacias with native species are an important component of the recognised suite of factors including introduction history, human use and enemy release that combine to produce successful invasions. Traits interact to confer Australian acacias with the relative competitive advantage over native species. One such interaction is that of N_2 fixation, which when coupled with slow decomposition of sclerophyllous phyllodes results in alteration of soil nutrient cycling. The lasting legacy of soil N-

enrichment hinders the competitive ability of native species and further enhances invasions. The importance of edaphic factors and competitive interactions in determining invasive success should be considered in predictive modelling of species distributions

4.2. Introduction

The ability of alien species to establish, grow and survive in non-native environments is highly dependent on their ability to compete with native species for the essential resources of light, water and nutrients. Australian acacias (1029 species in the subgenus *Phyllodineae* DC native to Australia; Miller *et al*., 2011; Richardson *et al*., 2011) are some of the most successful and prolific invasive species globally (Lowe *et al*., 2004; Henderson, 2007; Richardson & Rejmánek, 2011). Invasive Australian acacias successfully compete for resources in non-native environments (Werner *et al*., 2008), enabling them to realise their potential to grow larger than native vegetation (Table 4.1). Success of invaders is also contingent on several other factors (Rejmánek *et al*., 2005; Thuiller *et al*., 2006). For example, the history of introduction (both frequency and magnitude) and the human use of the introduced species play an important role in the subsequent scale of invasion (Lockwood *et al*., 2005; Wilson *et al.*, 2007; Caruthers *et al*., 2011; Griffin *et al*., 2011; Kull *et al*., 2011; van Wilgen *et al*., 2011). Reproductive and dispersal strategies can also influence the likelihood of invasion success (Gibson *et al*., 2011). Furthermore, the absence of alien species' pests and pathogens in a new host range also enhances the competitiveness of alien species (Williamson, 1996; Crawley, 1997). In this article, however, we focus on identifying ecophysiological traits and mechanisms enabling acquisition and conservation

of resources that contribute to competitive success of Australian acacias in non-native ranges.

Table 4.1. Height, aboveground biomass and Normalized Difference Vegetation Index (NDVI) of invasive Australian acacia stands in comparison to native vegetation in the Cape Floristic Region (CFR), South Africa. Values indicate means ± SE, where available**.**

***Acacia* spp.**	**Measure**	**Acacia**	**Native**	**Reference**
A. saligna	Height (m)	6.0	2.5	1
A. cyclops & A. saligna	Biomass (kg m^{-2})	10.4	2.0-3.5	2
A. saligna	Biomass (kg m^{-2})	5.8	1.8	1
A. cyclops & A. saligna	NDVI	0.63 ± 0.01	0.51 ± 0.01	3

[1]van Wilgen & Richardson, 1985; [2]Milton & Siegfried, 1981; [3]Fatoki, 2007.

Of the *ca.* 300 Australian *Acacia* spp. introduced around the world 23 have become highly invasive (Richardson & Rejmánek, 2011), particularly in Mediterranean-type ecosystems such as the South African Cape Floristic Region (CFR) or Portuguese dune ecosystems (Groves & di Castri, 1991; Witkowski, 1991a; Stock *et al*., 1995; Marchante *et al*., 2003; Rouget *et al.*, 2003; Werner *et al*., 2010) which are often water and/or nutrient limited. The effects of Australian acacia invasions on native ecosystems have been widely documented with a range of recorded impacts (reviewed in van Wilgen *et al*., 2008; Le Maitre *et al*., 2011) including declines in native species diversities (Richardson *et al*., 1989; Holmes & Cowling, 1997; Marchante *et al.*, 2003), reductions in stream flows due to increased water use (Enright, 2000; Dye *et al*., 2001), alterations to nutrient cycling (Yelenik *et al*., 2004) and modifications to fire regimes (van Wilgen & Richardson, 1985). Many of these impacts are linked with the propensity of the Australian acacias to grow much larger, in both height and total biomass, than the native vegetation in invaded ranges (e.g. Table 4.1). The vigorous vegetative growth of these plants also supports prolific

production of nutrient-rich seeds, leading to large, persistent seed banks (Milton, 1980; Holmes, 1989; Gibson *et al*., 2011) which are a major factor contributing to their successful invasion and persistence (Richardson & Kluge, 2008).

Competition for the resources to support this growth capacity depends on characteristics of both the invaded region and the invader's biological traits (Thuiller *et al*., 2006). Disturbance and the native plant community matrix in the invaded region strongly influence the distribution and availability of resources to alien plants. The "Empty Niche Hypothesis" suggests that alien plants are able to establish, persist and invade in novel environments by accessing resources not utilised by native flora (Elton, 1958; MacArthur, 1970). This was expanded upon by Davis *et al*. (2000) in the "Fluctuating Resource Hypothesis", which proposes that invasions are facilitated only when resources fluctuate (due to excess inputs into the system or reduced use by native flora) and temporarily become available for acquisition by invasive species.

Whether the invader can capitalise on the availability of resources is governed by its biological traits. Researchers have, over several decades, investigated which biological traits confer success of invaders over native species (Baker, 1974; Rejmánek & Richardson, 1996; Pyšek & Richardson, 2007). One key trait commonly recognised to support successful invasions is the ability of aliens to better acquire limiting resources or to use resources more efficiently than native species (e.g. Vitousek, 1986; Cordell *et al*., 2002; Funk & Vitousek, 2007). Here we review key ecophysiological traits and mechanisms that enable invasive Australian acacias to acquire the resources (light, water and nutrients) necessary to support the greater vegetative growth and meet the reproductive costs associated with successful invasions.

4.3. Resource acquisition and use by invasive Australian Acacias

4.3.1 Light

Competition for light is likely to be most greatest during germination. Invasive Australian acacia seedlings grow taller (*Acacia mangium* 50% taller than a common heath-forest species in Borneo, *Melastoma beccarianum*, Osunkoya *et al*., 2005; *A. saligna* 123% taller than a fynbos biome species in South Africa, *Protea repens*, Witkowski, 1991b) and faster than native species (Witkowski, 1991b; Peperkorn *et al*., 1995; Osunkoya *et al*., 2005). Once established, the fast-growing Australian acacias overtop native vegetation, outcompeting native species for light (Rutherford & Bösenberg, 1988). As a consequence, native vegetation most often cannot survive under the dense invasive Australian acacia canopies, leaving the understory bare (Holmes & Cowling, 1997). If native vegetation is able to persist, it is often only at the acacia canopy edge or at low levels in the understory (Midgley *et al.*, 1992). The high growth rates of Australian acacias are most likely supported by the superior abilities of Australian acacia seedlings to obtain necessary water and nutrients.

4.3.2 Water

Water depletion in invaded ecosystems is considered one of the most significant impacts of Australian acacia species (Le Maitre et al., 1996; 2000; Enright, 2000; Le Maitre, 2004). Several studies provide evidence for increased water use by Australian acacias in invaded regions. In a Portuguese pine forest, stand water use increased by 6.5% due to *Acacia longifolia* invasions (Table 4.2). Evapotranspiration (ET) was 13-51% higher in *A. mearnsii* stands compared to native vegetation in southern African grassland and Cape

Floristic Region (CFR) sites (Table 4.2.). Furthermore, invasive Australian acacias decreased the water yield of sampled South African river catchments by up to 5% (Table 4.2), a value predicted to increase dramatically with the projected spread of invasions (Le Maitre et al., 2002). Increased water use is likely a result of larger above-ground biomasses (ca. 3-fold greater) of Australian acacia stands compared to native vegetation (Table 4.1). Larger above-ground biomass yields an associated higher leaf area for transpiration as indicated using Normalized Difference Vegetation Index data (NDVI) as a proxy for leaf area index (Turner et al., 1999), which was 25% greater in invasive Australian acacia stands compared to native CFR vegetation (Table 4.1).

Apart from water loss due to greater above-ground biomass, leaf-specific water loss (i.e. transpiration rates) of invasive Australian acacias is also an important consideration, although relatively poorly documented. Rutherford & Bösenberg (1988) reported that A. cyclops generally had higher transpiration rates per leaf area than indigenous fynbos species. Further studies would thus be useful in determining whether increased water use is mostly due to larger above-ground biomass or also partially due to increased transpiration rates per leaf area. What is of interest is how invasive Australian acacias access these significant volumes of water.

Table 4.2. Effect of Australian acacia invasions on stand level water-use (scaled up from individual tree sap flows derived using Granier's constant heat method), modelled evapotranspirations derived using the Bowen ratio energy balance technique, and estimated water yields of sampled catchment areas (based on biomass-based regression models) when compared to native uninvaded vegetation.

***Acacia* spp.**	**Country**	**Vegetation type**	**Measurement**	**Effect**	**Reference**
A. longifolia	Portugal	Pine forest	Stand water-use	6.5% increase	1
A. mearnsii	South Africa	CFR vegetation	Evapotranspiration	13% increase	2
A. mearnsii	South Africa	Grassland	Evapotranspiration	51% increase	2
Acacia spp.*	South Africa	Native vegetation	Water yield of catchment (m^3)	1-5% reduction	3

[1]Rascher *et al*., 2009; [2]Dye *et al*., 2001; [3]Le Maitre *et al*., 2002.

*Mixed species including *A. cyclops, A. dealbata, A. longifolia, A. mearnsii, A. melanoxylon* and *A. saligna*.

Table 4.3. Comparison of root mass ratios (RMR), root biomass and root length indicated by a ratio of the measure comparing invasive Australian acacia seedlings to co-occurring native species in invaded ranges. Data are for vegetation varying from 6-18 months in age.

***Acacia* spp.**	**Region**	**Native spp.**	**Vegetation age (months)**	**RMR**	**Root biomass**	**Root length**	**Reference**
A. longifolia	Portugal	*Halimium halimifolium*	7	1.5x	3.0x	1.6x	1
		Pinus pinea		0.7x	0.9x	4.1x	
A. saligna	CFR	*Protea repens*	6	1.6x	15.0x	1.8x	2
A. saligna	CFR	Ericoid spp.	18	1.6x	3.3x	1.7x	3
		Restioid spp.		0.5x	1.8x	1.9x	
		Proteoid spp.		1.5x	2.5x	1.5x	

[1]Peperkorn *et al*., 2005; [2] Witkowski, 1991b; [3]Musil, 1993

4.3.2.1 Water acquisition

Plant water acquisition is dependent on the size, surface area and depth of its roots as well as how these roots are spatially distributed though the soil profile (Shenk & Jackson, 2002). Invasive Australian acacias generally have a higher investment in rootstocks (measured by root mass ratio; RMR) than native species, yielding a higher biomass of roots (Table 4.3) with a surface area 2- to 6-fold greater than that of native species (Werner *et al*., 2010). Australian acacia seedlings also develop roots 1.5- to 4-fold longer than co-occurring native species (Table 4.3), which penetrate deeper into the soil profile (Witkowski, 1991b). This occurs at significantly faster rates than that of native vegetation with no associated reduction in above-ground biomasses (Witkowski, 1991b; Musil, 1993; Peperkorn *et al*., 1995). The substantial and rapid root growth of acacia seedlings enables these plants to outcompete native species for water, especially during water-limited periods, a trait that has also been recognised as an advantage for other invasive species (Roché *et al*., 1994). Data on rooting patterns of larger, mature Australian acacias are sparse. However, considering the substantial initial root investment and strong correlations between above-ground and below-ground biomass of trees (e.g. Robinson, 2004), it is likely that large adult invasive Australian acacias also have substantially greater root investment than native species. One study described how *A*. *saligna,* when grown in a mixed stand with *Eucalyptus* and *Atriplex* had roots as deep as 6 m within four years of planting and at one site had roots up to 16 m deep after several additional years (Knight *et al*., 2002). Further information about rooting depth is imperative for understanding the

capacity of invasive Australian acacias to access deep water (and associated nutrient sources) possibly facilitating competitive exclusion of native species.

The spatial distribution of root biomass is also of great importance in determining the success of sustained water acquisition. A dimorphic root system comprising both deep roots enabling water acquisition during dry periods and a dense shallow network of surface roots that obtain water from the upper soil horizon in wetter periods is of great benefit (Pate *et al*., 1995; Canadell *et al*., 1996, Joffre *et al*., 2007). Juvenile *A. saligna* and *A. cyclops* show dimorphic roots in the invaded CFR, South Africa (Hoffman & Mitchell, 1986), whereas the native Fabaceae species compared, *Aspalathus albens*, *A. flexuosa* and *Rafnia angulata*, have significantly fewer surface lateral roots, with no lateral roots at all in the dry summer (Hoffman & Mitchell, 1986).

Overall, invasive Australian acacias show substantial initial below-ground investment producing bigger root systems that penetrate deeper into the soil in comparison to native species. Both the size and the spatial distribution of roots provide an early competitive advantage for water acquisition, particularly in water-stressed periods.

4.3.2.2 Water use efficiency

Water-use efficiencies (WUE) of invasive Australian acacias do not show significant differences in comparison to native vegetation in invaded areas (instantaneous gas exchange measures and long-term measures using foliar $\delta^{13}C$ ratios as a proxy; Table 4.4). The WUE's of invasive Australian acacias may however be situation and species dependent, particularly when considering the differences in foliar types. One of the distinguishing

characteristics of majority of the Australian acacias (*ca.* 95%; Maslin & Stirton, 1997) is the fact that different foliar types exist between seedling and adult life stages (termed heteroblasty). Seedlings develop bipinnate compound leaves, which are replaced within weeks to years of emergence by modified petioles that enlarge and flatten to form simple leaf-like structures termed phyllodes (Walters & Bartholomew, 1984; Boland *et al*., 2006). In water-limited conditions when a higher WUE would be desirable, phyllodes may be of particular advantage as these are thought to confer tolerance to drought due to their sclerophyllous nature (Elias, 1981; Pasquet-Kok *et al.*, 2010). Additionally, the sensitivity of stomatal closure in phyllodinous Australian acacias in response to increased vapour pressure deficits could contribute to phyllodes being more drought-tolerant in water-limited areas (Ullmann, 1989; Brodribb & Hill, 1993; Pasquet-Kok *et al.*, 2010). Low WUE's on the other hand, would exist when water is abundant and transpiration can occur freely. Thus, WUE is likely to be highly plastic. However, available data are from contexts in which water was readily available and hence marked differences between the WUE's of Australian acacias and native species cannot be expected. Thus, investigations into the response of WUE's of Australian acacias in comparison to native species under varying water availabilities are still needed.

Table 4.4. Instantaneous water-use efficiency (WUE) measured by gas exchange analysis and long-term WUE determined from foliar $\delta^{13}C$ of invasive Australian acacias in comparison to native species. Data collected by Kraaij & Cramer (1999) are from a non-riparian field site in the high-rainfall season (Aug-Sep 1998) in the Cape Floristic Region (CFR), South Africa. Peperkorn *et al*. (2005) provide data from a greenhouse study in which plants were irrigated. Data derived from Crous (2010) are from riparian field sites located in the CFR, South Africa. Relative to natural vegetation, + indicates a higher WUE of Australian acacias, 0 indicates no significant difference and – indicates a lower WUE.

***Acacia* spp.**	**Native spp.**	**Water availability**	**Measure**	**Comparison**	**Reference**
A. longifolia	*Protea repens*	High – wet season	WUE	0	1
	Chrysanthemoides monilifera	High – wet season	WUE	+	1
	Dodenea viscosa	High – wet season	WUE	0	1
	Leucadendron salignum	High – wet season	WUE	+	1
A. longifolia	*Halimium halimifolium*	High – irrigated	WUE	0	2
A. mearnsii	*Brabejum stellatifolium*	High – riparian	$\delta^{13}C$	0	3
	Metrosideros angustifolia	High – riparian	$\delta^{13}C$	–	3
A. saligna	*Protea repens*	High – wet season	WUE	0	1
	Chrysanthemoides monilifera	High – wet season	WUE	0	1
	Dodenea viscosa	High – wet season	WUE	0	1
	Leucadendron salignum	High – wet season	WUE	+	1

[1]Kraaij & Cramer, 1999; [2]Peperkorn, *et al*., 2005; [3]Crous, 2010

4.3.3. Nutrition

Alien plant invasions can occur in a range of nutritional environments including infertile environments (Funk & Vitousek, 2007). This is also true of Australian acacias, which are often highly competitive in nutrient-poor Mediterranean-type ecosystems such as the CFR and Portuguese dune systems (Groves & di Castri, 1991). Invasive Australian acacias are able to effectively acquire nutrients and have been shown to have greater leaf N concentrations than native species in invaded regions, while P concentrations are slightly more variable (Table 4.5). Musil (1993) found that *A. saligna* also exhibited greater

concentrations of K, Ca and Mg than native fynbos species. Considering that Australian acacias themselves originate from some of the most nutrient-poor soils in the world (Young & Young, 2001) it is not surprising that these species are able to effectively compete for nutrients, leading us to question whether these plants possess particular traits or mechanisms that enhance their competitive ability for nutrient acquisition and conservation.

Table 4.5. Foliar N and P concentrations (mean mg g^{-1} ± SE) of Australian acacia species in comparison to native species from the invaded region. Significantly larger values ($P < 0.05$) in comparisons are in bold. ND indicates no available data. Available data stem from studies in the Cape Floristic Region (CFR), South Africa.

***Acacia* spp.**	**[N]**	**[P]**	**Native spp.**	**[N]**	**[P]**	**Reference**
A. cyclops	**18.5 ± 0.71**	2.58 ± 0.10	*Pterocelastrus tricuspidatus*	9.70 ± 0.26	**6.31 ± 0.47**	1
			Protea repens	2.38 ± 0.56	ND	2
A. longifolia	**9.94 ± 0.98**	ND	*Chrysanthemoides monilifera*	4.06 ± 0.42	ND	2
A. saligna	**13.31 ± 2.66**	ND	*Dodenea viscosa*	5.04 ± 0.56	ND	2
			Leucadendron salignum	1.82 ± 0.28	ND	2
A. saligna	**20.50 ± 0.77**	1.01 ± 0.07	*Leucospermum parile*	7.90 ± 0.31	0.93 ± 0.04	1
A. saligna	25.00 ± 3.57	**1.48 + 0.16**	*Protea repens*	18.00 ± 2.04	0.65 + 0.21	3
			Ericoid	12.75 ± 0.7	0.59 ± 0.06	4
A. saligna	**14.29 ± 0.14**	**0.7 ± 0.01**	Restioid	7.56 ± 0.14	0.31 ± 0.03	4
			Proteoid	8.82 ± 0.42	0.59 ± 0.03	4

[1]Witkowski, 1991a; [2]Kraaij & Cramer, 1999; [3]Witkowski, 1991b; [4]Musil, 1993

4.3.3.1. Nutrient acquisition

Nutrient acquisition by plants is influenced by three major factors: root structure (including biomass, surface area and spatial distribution), soil nutrient availability, and the ability of the plant to form specialised associations for nutrient acquisition (Lambers *et al*., 2008a). Root biomass can be preferentially allocated to enriched shallow soils and/or towards growing deeper roots in order to tap unused nutrient resources (Jobbágy & Jackson, 2001; Lambers *et al*., 2008a; Craine, 2009). As discussed above, invasive Australian acacias allocate a greater percentage of biomass to both deep and shallow roots in comparison to native species in invaded regions (Table 4.3). Acacia roots may also be more plastic in response to soil nutrient availability than other species. For example, the RMR of *A. longifolia* almost doubled when nutrient concentrations were reduced, compared to native Mediterranean dune species *Halimium halimifolium* and *Pinus pinea* (Peperkorn *et al*., 2005).

The availability of soil nutrients to a plant is dependent on soil moisture and the ability of the plant to increase available nutrient concentrations through the use of root exudates. Soil moisture strongly influences the diffusive flux of nutrients into the rhizosphere. Plants can alter soil moisture by redirecting available water resources via hydraulic redistribution (Burgess *et al*., 1998; Hawkins *et al*., 2009), potentially increasing the solubility and hence availability of nutrients to plant roots (Jackson *et al*., 2000; Ryel, 2004; Hawkins *et al*., 2009). However, very little direct evidence for nutrient acquisition via hydraulic redistribution is available (Lambers *et al*., 2006) and this remains an untested possibility for invasive Australian acacias. Furthermore, transpirational water use by plants also drives nutrient mass flow (Barber, 1995) and transpiration is thus partially regulated

by nutrient availability, particularly N (Raven *et al.*, 2004; Cramer *et al*., 2008; 2009; Cernusak *et al*., 2010). Mass-flow of nutrients requires adequate soil water to supply transpirational demand and hence operates at the expense of WUE (Barber, 1995; Tinker & Nye, 2000; Raven *et al.*, 2004; Cramer *et al*., 2009). For many species a decrease in nutrient availability decreases WUE (Raven *et al.*, 2004), as has also been observed for *A. longifolia* (Peperkorn *et al*., 2005) suggesting that a water-nutrient trade-off may occur. The fact that water is required for both diffusive and mass-flow mobility of nutrients in soil provides a powerful explanation for the interaction of these two resources in determining plant growth.

Soil nutrient concentrations can also be altered by plants actively extracting nutrients that are not readily available, through the release of root exudates such as carboxylates and phosphatases (Lambers *et al*., 2008a). In the highly invaded South African CFR, well represented families such as Proteaceae and the Restionaceae commonly produce specialised cluster roots, which increase surface area for diffusion and exudate release (Lamont, 1982; Lambers *et al*., 2006). Cluster roots are efficient at acquiring nutrients, particularly P from low-concentration and sparingly soluble sources (Lambers *et al*., 2006). Invasive Australian acacias lack cluster roots and are thus unlikely to be able to access these more recalcitrant forms of soil P. Despite this, invasive Australian acacias still compete effectively for nutrients in the intrinsically nutrient-poor soils of the CFR (Table 4.5). This competition may be enhanced through the ability of plants to form symbiotic mycorrhizal associations for nutrient acquisition (Lambers *et al*., 2008a).

Mycorrhizal associations occur in 82% of higher land plants (Brundrett, 2002) and enhance nutrient (particularly P) acquisition (Lambers *et al*., 2008b, Smith & Read, 2008).

Both arbuscular mycorrhizas (AM) and ectomycorrhizas (EM) are able to take up soluble P from the soil, but only EM are able to chemically release P from sorbed and organic complexes (Smith & Read, 2008). Most Australian acacia species are able to form AM and possibly also EM associations (Reddell & Warren, 1987). However, the relative importance of these associations for P uptake in acacias remains unclear. Hoffman and Mitchell (1986) showed a positive correlation between AM colonisation with plant biomass accumulation and P content of *A. saligna* seedlings in the CFR. In contrast, Rodríguez-Echeverría *et al*. (2009) found that despite significant colonisation of *A. longifolia* roots by AM fungi in Mediterranean dune systems, no advantage in P acquisition was conferred. The benefits of EM and AM associations in Australian acacias must depend on the form and availability of P in the soil. The formation of mycorrhizal associations and the lack of cluster roots are likely to restrict the invasive Australian acacias to dependence on organic P and the more soluble forms of inorganic P. This inability to acquire the sparingly soluble forms of P that cluster-rooted species (particularly Proteaceae, Restionaceae and Fabaceae) of the invaded CFR do may serve to limit invasions of Australian acacias on some extremely nutrient-impoverished sandstone-derived soils of the CFR.

Australian acacias are well known for their N_2-fixation abilities (Levine *et al*., 2003). N_2-fixing associations occur in most Australian acacias (Lawrie, 1981; Lee *et al*., 2006), which usually nodulate with common, but slow-growing *Bradyrhizobium* species (Lafay & Burdon, 2001; Rodríguez-Echeverría *et al*., 2011). Associations with other nodulating species have also been reported, including *Rhizobium*, *Ensifer*, *Mesorhizobium*, *Burkholderia*, *Phyllobacterium* and *Devosia* species (Marsudi *et al.*, 1999; Lafay & Burdon, 2001; Hoque *et al*., 2011). Associations between acacias and their nodulating symbionts are highly complex

and can be influenced by several biotic and abiotic factors (Thrall *et al*., 2000; Murray *et al*., 2001; Thrall *et al*., 2007; Rodríguez-Echeverría *et al*., 2011). Nonetheless, invasive Australian acacias nodulate readily in both their native and non-native regions (reviewed in this volume by Rodríguez-Echeverría *et al*., 2011) and are considered prolific N_2-fixing species (Lawrie, 1981). In coastal dunes of Portugal, *A. longifolia* was more efficient at forming symbiotic associations with bacteria and fixed greater amounts of N than other co-occurring N_2-fixing legumes (*Ulex eurpaeus* and *Cytisus grandiflorus*; Rodríguez-Echeverría *et al*., 2009). Similarly, comparing the $\delta^{15}N$ of N_2-fixing plants to others with N_2 fixation disrupted by O_2 fumigation, Stock *et al*. (1995) found that *A. saligna* in the CFR relied almost completely on symbiotic N_2 fixation, while *A. cyclops*, growing on slightly more nutrient-rich soil obtained only 51% of its N budget from N_2 fixation. The long-term post-fire persistence of invasive Australian acacias in the CFR is somewhat puzzling because few native N_2-fixing legumes (especially reseeders) persist beyond their post-fire dominance (Kruger, 1983; Hoffmann *et al.*, 1987; Cocks, 1994; Cramer, 2010). This lack of indigenous legume reseeder persistence has been ascribed to the post-fire decline in P-availability (Power *et al*., 2010). These authors suggested that deep roots and excessive water consumption may contribute to Australian acacia persistence.

The N_2-fixing capabilities of Australian acacias and their ability to persist in invaded regions results in a substantial inputs of N-enriched litter, leading to an elevated soil N status (Table 4.6). However, the ability of an invader to fix N_2 in itself does not necessarily translate to immediate alteration of the invaded system's nutrient cycling (Corbin & D'Antonio, 2004). Instead, Yelenik *et al*. (2007) demonstrated that with Australian acacias, the combination of N_2 fixation coupled with the slow decomposition rates associated with

sclerophyllous phyllodes led to elevated soil N pools with long-term impacts for ecosystem nutrient cycling. Australian acacias are thus strong ecosystem engineers and the lasting legacy of increased soil N following Australian acacia invasion often results in reinvasion by the same or other alien species (Stock *et al*., 1995; Yelenik *et al*., 2004; Marchante *et al*., 2004; 2008; 2009).

Table 4.6. Litter biomass, litter N concentrations and soil N concentrations of Australian acacia invasions compared to uninvaded native vegetation in Portugal and in the Cape Floristic Region (CFR). Data for longer (20+ years) and shorter (10 years) invasion periods are shown for Portugal. A + indicates a significantly ($P < 0.05$) greater value associated with Australian acacias in comparison to native vegetation, 0 indicates no significant difference and ND indicates no data available.

		Litter		Soil	
***Acacia* spp.**	**Region**	**Biomass**	**[N]**	**[N]**	**Reference**
A. cyclops	CFR	+	+	+	1
	CFR	ND	ND	+	2
A. longifolia	Portugal (20+ yrs)	+	+	+	3
	Portugal (10 yrs)	+	+	0	3
	Portugal	ND	ND	+	4
A. saligna	CFR	+	+	+	1
	CFR	ND	ND	+	5
	CFR	ND	ND	+	2
	CFR	+	+	+	6

[1]Witkowski, 1991b; [2]Stock *et al*., 1995; [3]Marchante *et al*., 2008; [4]Rodríguez-Echeverría *et al*., 2009; [5]Musil, 1993; [6]Yelenik, 2004;2007.

5.3.3.2 Nutrient conservation

The sclerophyllous nature of Australian acacia phyllodes translates to long-lived leaves and evergreen trees (Loveless, 1961; Turner, 1994a). The evolutionary drivers for this adaptation, whether drought tolerance or nutrient conservation, have been subject to much debate (Givnish, 1979; Turner, 1994b; Pasquet-Kok *et al*., 2010). In nutrient-rich

environments the common drought-tolerance adaptation is drought deciduousness (Mooney & Dunn, 1970). However, in nutrient-poor environments drought deciduousness would lead to the costly loss of limited nutrients. Thus, it is thought that sclerophyllous, long-lived phyllodes evolved in order to enhance nutrient conservation in response to nutrient limitations (Beadle, 1966; Specht & Rundel, 1990) with drought tolerance and unpalatability being associated with the sclerophyllous nature of phyllodes.

Extended leaf longevity of Australian acacias would, however, likely not provide a marked advantage when invading other sclerophyllous vegetation with similar nutrient-retention characteristics. For example leaf longevity of Australian acacias (mean years ± SE; 1.84 ± 0.28; Wright *et al*., 2002) did not differ significantly ($P > 0.05$) from that of native CFR vegetation (mean ± SE; 2.62 ± 0.31; Midgley & Enright, 2000). Sclerophylly, although not different from that of the invaded flora, when coupled with other traits such as N_2 fixation may contribute to the success of Australian acacias. Interestingly the non-phyllodinous and relatively non-sclerophyllous (i.e. high SLA) invasive Australian acacia, *A. mearnsii*, has particularly long-lived bipinnate leaves, which turn brown during drought but recover subsequent to the onset of rain (Orians & Milewski, 2007) possibly acting to conserve nutrients over multiple seasons.

Heteroblasty thus confers the advantage of different growth strategies between juvenile and adult life stages and between different environmental circumstances (Pasquet-Kok *et al*., 2010). As young seedlings, acacias benefit from the high relative growth rate associated with bipinnate leaflets (Hansen, 1996; Witkowski, 1991b; Evans *et al*., 2000; Pasquet-Kok *et al*., 2010). The phyllodinous species then switch to slower-growing, longer-lived and hence nutrient-conserving phyllodes (Ullmann, 1989; Orians & Milewski, 2007;

Pasquet-Kok *et al.*, 2010). Using acacia invasions in South Africa as a case study, the distinct advantage of phyllodes in nutrient-poor and summer-drought regions can be inferred by the relative success of phyllodinous species in the Mediterranean-climate and nutrient-poor fynbos biome (Rouget *et al.*, 2004; Table 4.7, e.g. *A. pycnantha*). In contrast, the non-phyllodinous species (e.g. *A. mearnsii* and *A. dealbata*) are more successful as invaders in more mesic environments or along water courses (Rouget *et al*., 2004; Table 4.7) where nutrients and water are not as limiting.

Plants can also conserve nutrients through the remobilisation of limiting nutrients prior to leaf abscission (Eckstein *et al*., 1999; Wright *et al*., 2002), acting to increase the mean residence time of nutrients in the plant. In the South African CFR, *A. saligna* remobilised a large proportion (71%) of its leaf P, an amount significantly greater than that of the comparison native species *Leucospermum parile* (48%; Witkowski, 1991a). However studies assessing remobilisation efficiencies of these plants in comparison to natives in invaded regions are scarce. Specht (1981) and Langkamp & Dalling (1982) showed that remobilisation of nutrients by invasive Australian acacias was not particularly different to that of other Australian species from nutrient impoverished areas (e.g. *Banksia ornata* and *Acacia holosericea*) and is thus not a trait unique to the invasive Australian acacias.

Table 4.7. Percentage of records of the eight most widespread invasive Australian acacia species found in each biome in South Africa. Species are ranked from most prevalent to least prevalent according to the percentage of quarter degree squares occupied, as recorded in the South African Plant Invaders Atlas (SAPIA; Henderson, 2007). The percentage of the total records that were found along water courses is also listed. Foliage indicates whether adult plants have leaves (L) or phyllodes (P). The biome in which each species had the highest occurrence is in bold.

			Percentage of records found in each biome*					
***Acacia* spp.**	**Foliage**	**QDS (%)**	**Savanna**	**Fynbos**	**Grass-land**	**Nama karoo**	**Succulent karoo**	**Water courses**
A. mearnsii	L	21	27	33	**38**	0	1	36
A. dealbata	L	12	12	2	**85**	0	0	50
A. saligna	P	8	9	**83**	0	0	7	35
A. cyclops	P	8	16	**74**	0	0	10	19
A. melanoxylon	P	7	15	**62**	23	0	0	27
A. longifolia	P	5	18	**73**	9	0	1	36
A. decurrens	L	5	16	0	**84**	0	0	13
A. pycnantha	P	2	3	**97**	0	0	0	4

* Biomes classified according to Rutherford, 1997.

4.4. Implications for future invasion risk

Global change is expected to alter resource distribution and availabilities through changes to climate, nutrient cycling (through nutrient deposition), disturbance regimes and land-use practices. These changes are generally predicted to favour the increase and impacts of alien plant invasions around the globe (Dukes & Mooney, 1999; Thuiller *et al*., 2007; Vilà *et al*., 2007), although there are exceptions (Richardson *et al*., 2000; Richardson *et al*., 2010). Invaders may be able to benefit from these changes either by being stronger competitors for resources or due to decreased competition from stressed native species (Thuiller *et al*., 2007), as suggested by Davis *et al*. (2000) in the "Fluctuating Resource Hypothesis". Under elevated [CO_2] environments, Australian acacias have higher net assimilation rates, leading to increased relative growth rate and plant biomass (Table 4.8).

Plants also showed a decrease conductance and hence increased WUE (Table 4.8). Moreover, Australian acacias also fixed greater amounts of N_2 under elevated [CO_2] (Table 4.8) as has also been shown for several other N_2-fixing species (Thomas *et al*., 1991; Vogel & Curtis, 1995; Polley *et al*., 1997). These studies indicate that with continuing global change, Australian acacia species may well have increased invasive competitive abilities for resource acquisition, further enhancing their invasion success.

Table 4.8. Response of net assimilation rate (NAR), relative growth rate (RGR), total plant biomass, stomatal conductance, and total N fixed of invasive Australian acacias under elevated CO_2 levels (700 ppm). Foliage indicates whether adult plants have leaves (L) or phyllodes (P). + indicates an increase, 0 indicates no significant difference and – indicates a decrease in comparison to current ambient CO_2 levels (350 ppm), while ND is no data available.

***Acacia* spp.**	**Foliage**	**NAR[1,2]**	**RGR[1,2]**	**Biomass[1,2]**	**Conductance[3]**	**Total N fixed[2]**
A. dealbata	L	+	+	+	0	0
A. implexa	P	+	+	+	+	+
A. irrorata	L	+	+	+	–	+
A. mearnsii	L	+	+	+	–	+
A. melanoxylon	P	+	+	+	–	+
A. saligna	P	+	+	+	0	ND

[1]Atkin *et al*., 1999; [2]Schortemeyer *et al*., 2002; [3]Evans *et al*., 2000

4.5. Conclusion

To synthesise, the height and biomass of invasive Australian acacias in invaded ranges far exceeds that of the native species both as seedlings and as adults. Initial high relative growth rates allow acacias to overtop the native vegetation and outcompete natives for light. Greater below-ground investment combined with mycorrhizal and N_2-fixing symbioses enable access to both water and nutrients needed to sustain growth.

Furthermore, sclerophylly and the greater ability to remobilise limiting nutrients enables efficient nutrient conservation. Thus, no one ecophysiological or morphological trait can be identified as the primary driver of invasion success. Instead it would appear that multiple traits act synergistically to confer competitive advantage. Understanding the traits used by invasive Australian acacias to acquire, utilise and conserve essential resources will allow us to better understand how resource distribution and availability influence invasions across a landscape. Incorporating greater edaphic and biotic components of the invaded environments into current species distribution models would thus enhance predictive power of models which are currently mostly limited to the use of only abiotic factors and limited soil characteristics (Meier *et al.*, 2010). This is vital for predicting alien plant distributions, both under current and future global change scenarios.

CHAPTER 5
THE ECOPHYSIOLOGICAL TRAITS ASSOCIATED WITH RESOURCE ACQUISITION OF ADULT *ACACIA CYCLOPS* IN STRANDVELD VEGETATION OF THE CAPE FLORISTIC REGION

5.1. Abstract

Tree invasions are recognized to pose a significant threat to the biodiversity and ecosystem functioning of Mediterranean-type ecosystems around the globe. It is suggested that in order to successfully invade these characteristically resource poor environments, invasive species may better acquire available resources not readily accessible to native species or use available resources more efficiently than native species. My study aimed to assess the ecophysiological traits associated with invasion success of the invasive Australian species, *Acacia cyclops* in coastal vegetation of the South African Mediterranean-type ecosystem. To achieve this, I compared several ecophysiological traits associated with resource acquisition and use, between mature *A. cyclops* trees and three abundant native woody species of similar growth form in intact and undisturbed coastal vegetation. Results showed that *A. cyclops* was able to maintain high photosynthetic rates over both the dry summer and wet winter seasons (*ca.* 15 $\mu mol\ m^{-2}\ s^{-1}$) distinguishing performance of the invasive species in comparison to the native species. More negative δD values ($P < 0.001$) of *A. cyclops* (-43 ‰) in comparison to the native species (-29 to -37 ‰) may indicate deeper rooting abilities and sustained access to water resources by the invasive species over both the wet and the dry season that would support the high photosynthetic rates even in the dry summer season. Additionally, 30-50% greater foliar N concentrations ($P < 0.001$) of *A. cyclops* in comparison to the native species in conjunction with lower foliar $\delta^{15}N$ values, likely associated with N_2-fixation, suggest that *A. cyclops* is better able to

acquire N resources regardless of N availability in the soil. Thus invasive success of *A. cyclops* in the resource limited Mediterranean type ecosystem is likely more attributable to greater resource acquisition rather than through greater resource use efficiency as has been found for other invasive species.

5.2. Introduction

Mediterranean type ecosystems contribute almost 20% of the worlds known plant species despite covering only 5% of the land surface area (Cowling *et al.* 1996). These highly diverse ecosystems, found in South Africa, Australia, Europe, California and Chile, are however highly threatened by alien plant invasions (Gritti *et al*. 2006 Rejmánek and Randall 1994, Rouget *et al*. 2003, Seabloom *et al.* 2006, Underwood *et al.* 2009). Tree invasions are particularly prevalent (Rundel *et al.* 2014) and dense monospecific stands often result in widespread ecological impacts including reductions in biodiversity, alterations to disturbance regimes, changes to biogeochemical cycling and reductions in water availability (reviewed in Le Maitre *et al.* 2011; Richardson and Rejmánek 2011; Rundel *et al*. 2014). In order for introduced species to successfully invade and dominate these landscapes, it is suggested that a greater fitness advantage over native species is necessary (MacDougall *et al*. 2009; Rundel *et al*. 2014).

Fitness advantages through higher relative growth and photosynthetic rates have classically been associated with invasive success in high resource environments (Daehler 2003, Leishman *et al.* 2010, Leishman *et al.* 2007, Pysek and Richardson 2007). Recent studies suggest that these traits can also be associated with invasive success in resource limited environments (Funk and Vitousek 2007, Matzek 2011) and to do so it has been

suggested that invasive species must better acquire available resources, or use available resources more efficiently than native species (Funk 2013).

Under low soil nutrient conditions, resource acquisition traits such as high root:shoot ratios, fast root elongation rates, mycorrhizal associations and specialized root structure may aid in ameliorating nutrient limitations. While several of these traits have been associated with invasive species success, this is often context and species specific (reviewed in Funk 2013). One nutrient acquisition trait that is suggested to commonly facilitate the success of several invasive tree species in nutrient limited environments is the ability to acquire N through N_2-fixation (Rodríguez-Echeverría *et al.* 2009, Stock *et al.* 1995, Vitousek and Walker 1989, Yelenik *et al.* 2004). On the other hand, invasive plant species may use nutrients more efficiently under nutrient limited conditions and several invasive species have correlated greater performance of invaders with higher photosynthetic nutrient use efficiency (PNUE) in comparison to native species (reviewed in Funk 2013, Funk and Vitousek 2007, Matzek 2011).

In water limited environments, traits such as high root:shoot ratios or deeper rooting abilities may enable invasive species to better acquire scarce water resources, a trait that may be particularly important in enabling invasive species to tolerate summer drought conditions (reviewed in Funk 2013, Morris *et al.* 2011). Higher water use efficiency (WUE) could also be advantageous water limited environments. However several reviews and meta analyses have found little difference in instantaneous WUE or in measures correlated with integrated lifetimes WUE ($\delta^{13}C$) between invasive and native species (Cavaleri and Sack 2010, Funk 2013) suggesting that high WUE may not be an important trait facilitating invasion success.

The dry summer conditions and low soil nutrients, which characterize the Mediterranean-climate Cape Floristic Region (CFR) of South Africa (Specht and Moll 1983) are suggested to act as major limitations to tree growth (Rundel *et al.* 2014). Despite these limitations several Australian *Acacia* tree species of the subgenus *Phyllodineae* have become widely invasive in the region (Henderson 2007, Le Maitre *et al*. 2000). I hypothesized that in these low resource availability ecosystems, *A. cyclops* must exhibit ecophysiological traits allowing for greater resource acquisition and/or use efficiency that facilitate invasion success. To test this, I compared several ecophysiological traits of resource acquisition and use, between mature *A. cyclops* trees and three abundant native species of similar growth form in a Mediterranean shrubland ecosystem of the CFR.

5.3 Methods

5.3.1 Study sites and species

The study was conducted at three sites in the coastal "strandveld" vegetation type (Mucina and Rutherford 2006) in the Western Cape Province of South Africa, at Koeberg Nature Reserve (KB), De Kelders Nature Conservancy (DK) and the Vergaderingskop Nature Conservancy (VGK), where dense invasions of *A. cyclops* are known to occur. Strandveld vegetation grows on deep, well-drained marine-derived aeolian sands and is dominated by broad-leaved, sclerophyllous low stature (i.e. < 2 m) shrubs interspersed with succulents, bulbs and grasses (Mucina and Rutherford 2006). Although exact fire return intervals are unknown, fires are infrequent in strandveld ecosystems and are

estimated to occur every 50-200 years (Rebelo *et al*. 2006), providing a low disturbance system for the study.

The three sites receive similar annual precipitation (500 - 540 mm). This, however, is less strongly winter biased at Vergaderingskop, which is situated farther east than the other two sites (Table 1). Mean annual temperatures are 16 - 17°C, ranging between 7 - 26°C (average monthly minima – maxima; Table 1).

Table 5.1. Study site locations and climatic characteristics derived from data of Hijmans *et al.* (2005).

	Koeberg	De Kelders	Vegaderingskop
Coordinates	33.65287 S 18.43725 E	34.55466 S 19.37298 E	34.3579 S 21.56873 E
Mean annual temp. (°C)	16.5	16.2	17.1
Minimum temp. in coldest month (°C)	7.1	9.3	6.9
Maximum temp. in warmest month (°C)	26.4	23.1	26.2
Mean annual precipitation (mm)	533	540	500
Precipitation in coldest quarter (mm)	238	215	137
Precipitation in warmest quarter (mm)	54	65	99

At each site, a suite of ecophysiological measurements were collected on five replicates of the invasive, *A. cyclops*, and of three native co-occurring species including *Chrysanthemoides monilifera* (L.) Norlindh, *Euclea racemosa* Murray, and *Searsia laevigata* (L.). While comparisons of traits contributing to invasive success can often be most beneficial by comparing non-invasive to invasive congenerics, the lack of such comparison species in strandveld vegetation required comparisons to be made against the most appropriate species present. We chose native species with similar growth form and high prevalence in study sites (although *E. racemosa* was absent at KB). *Chrysanthemoides*

monilifera is a shrub growing to 3 m in height, *E. racemosa* is a shrub or small tree that grows to between 1 and 6 m tall and *S. laevigata* is a small shrub or tree growing 1 – 4 m tall (Coates-Palgrave 2002). *Acacia cyclops* is a phyllodinous, sclerophyllous, evergreen shrub growing to 4 – 6 m in height, native to coastal habitats of south-west Australia and was originally introduced into South Africa in the 19th century for dune stabilization (Poynton 2009).

5.3.2 Soil nutrient analyses

Twenty surface-soil cores (0 - 10 cm deep x 8 cm diameter) were collected under each plant replicate, from each site in April 2010. Samples were dried at 70°C for 48 h and passed through a 1-mm sieve to remove course organic matter. Total soil N was determined with a FP-528 Nitrogen Analyzer (Leco Corporation, St. Joseph, MI, USA). $\delta^{15}N$ was measured using mass spectrometry (Archeometry, University of Cape Town, South Africa). Milled samples were weighed into tin capsules and combusted in a Thermo Flash EA 112 series elemental analyzer coupled with a Delta Plus XP isotope ratio mass spectrometer (Thermo Electron Corporation, Milan, Italy) and an International Atomic Energy Authority standard was used to calibrate results. Total and available P were analyzed using Inductively Coupled Plasma Atomic Emission Spectroscopy (ICP-AES; Varian Vista MPX, Melbourne, Australia). Available P samples were prepared by extracting 6.6 g of soil in Bray II solution (Bray and Kurtz, 1945) and thereafter filtered. Exchangeable cations were displaced from 10 g soil with 25 mL of 0.2 M ammonium acetate and filtered samples were made to 200 mL and K, Na, Ca and Mg were measured using ICP-AES analysis.

5.3.3. Foliar nutrients

Foliar nutrient concentrations were compared between species. Ten young, fully expanded, unshaded leaves were collected in April 2010 and were bulked for each species (n = 5) at each site. Leaf samples were dried at 70°C for 48 h and ground in a Wiley mill to pass through a 1 mm sieve. Samples were chemically digested according to Castle and Neff (2009) and analyzed for P concentration by inductively coupled plasma optical emissions spectrometry/mass spectrometry (ICP-OES/ICP-MS). Nitrogen concentration, $\delta^{15}N$, and δ13C were measured by mass spectrometry (Archeometry, University of Cape Town, South Africa). The foliar $\delta^{15}N$ was used to assess whether plants were acquiring atmospheric N_2 through N_2-fixation (Lajtha and Marshal 1994). Since soil ^{15}N values are richer in ^{15}N than atmospheric N_2 (= 0 ‰), foliar $\delta^{15}N$ values of 0 (or below due to fragmentation during fixation) are generally indicative of the occurrence of N_2 fixation (Lajtha and Marshall 1994). Foliar $\delta^{13}C$, was used as an indicator of the ratio CO_2 concentration in the intercellular space of leaves to the CO_2 in the atmosphere (C_i/C_a ; Farquhar *et al*. 1982), which can provide information on patterns of stomatal limitation. Foliar samples were willed and weighed into tin capsules and combusted in a Thermo Flash EA 112 series elemental analyzer coupled with a Delta Plus XP isotope ratio mass spectrometer (Thermo Electron Corporation, Milan, Italy). An International Atomic Energy Authority standard was used to calibrate results.

5.3.4. Gaseous exchange measurements

Photosynthetic rate (A) and transpiration (E) were measured in dry summer season (April 2010) and in the wet winter season (July 2010) on one young, fully expanded, unshaded representative leaf of each species (n = 5) at each site. Photosynthetic rate was measured using a Li-Cor LI6400-40 fluorometer leaf chamber and Li-Cor LI-6400 infrared gas analyser (Li-Cor, Lincoln NE, USA). Measurements were taken over several days at each site, between 10:00 and 15:00 h local time. Photosynthetic flux density (PPFD), CO_2 concentration, and airflow within the leaf chamber were maintained at 1000 μmol m^2 s^{-1}, 400 ppm, and 150 μmol s^{-1}, respectively. The block temperature was set close to ambient temperatures, which was measured to be 25°C in summer (April) and 20°C in winter (July).

Instantaneous photosynthetic nitrogen use efficiency (PNUE = A/[foliar N]), photosynthetic phosphorus use efficiency (PPUE = A/[foliar P]) and water use efficiency (WUE = A/E) were calculated for each species (n=5) at each site for the summer season (April 2010).

5.3.5. Rooting depth

Stem xylem water δD (^{2}H:H ratio) was compared between species as a proxy of rooting depth, since direct determination of rooting depth of adult plants *in situ* was not possible. The isotopic composition of xylem water remains unaltered from roots to shoots during plant water transport (Ehleringer and Dawson 1992, White *et al.* 1985, Zimmermann *et al.* 1967). Stem xylem δD can thus be used to indicate water source of plants (Dawson and Ehleringer 1991, February *et al.* 2013). Additionally, because generally

δD of soil water becomes more negative with soil depth (Dawson and Ehleringer 1999), more negative xylem δD would indicate water acquisition from deeper soil depths from which the existence of deeper roots could be inferred. Stem segments approximately 8 cm long were cut from terminal branches of each species (n=3) at each site between 8:00 to 10:00 h in the summer (April 2010) and winter season (July 2010). Winter samples from VGK were not collected. Cut segments were immediately placed into glass Kimax tubes (Kimax-Kimble, Vineland, USA) and the seal reinforced with parafilm to prevent evaporation. Tubes were stored in a cooler box in the field and frozen upon return. Xylem water was extracted from stem segments by cryogenic vacuum distillation. δD was then determined at the University of Cape Town using a Finnigan Mat 252 mass spectrometer (Bremen, Germany). Results were expressed as parts per thousand deviations from the Vienna Standard Mean Ocean Water.

5.3.6 Statistical analyses

Response variables that were measured only once (soil nutrients, foliar nutrients, PNUE, and PPUE) were analyzed using a two-way ANOVA with species and site as factors. Response variables measured in both the summer and the winter season (A, E, WUE and δD) were analyzed using a three-way ANOVA with species, season and site as factors. Posthoc Tukey analyses were used to a determine significance of the main effects. Data that did not meet the assumptions of normality were log transformed before analysis. All analyses were performed using R-Project (R Core Team 2012).

5.4 Results

5.4.1 Soil nutrients

Soils sampled under each species did not differ from each other at any of the sites (Table 5.2). Instead, soils differed significantly between sites (Table 5.2). Koeberg had the lowest soil nutrient concentrations, followed by De Kelders and highest at Vergaderingskop for all soil nutrients except total and available P. Total P was highest at Koeberg, followed by Vergaderingskop and lowest at De Kelders, while available P at Koeberg was similar to Vergaderingskop and both sites had great Total P than De Kelders. Total N was similar or less than concentrations found in other CFR vegetation types. Total P at Koeberg and Vergaderingskop were at least double those of other CFR vegetation types, but despite this available P was similar if not slightly lower. Calcium in study sites was at least 2-fold greater than other CFR vegetation types although this is likely to be associated with the marine derived soils associated with coastal regions (Nyaga *et al*. 2013).

Table 5.2. Soil nutrient concentrations (mean ± S.E.) at Koeberg (KB), De Kelders (DK) and Vegaderingskop (VGK) (n=20). Soil nutrient characteristics from other CFR vegetation types are presented for comparison (Cramer *et al*. in press). F-values and significance from two-way ANOVA analyses are included and Tukey's posthoc differences are indicated with lower case lettering. Soil nutrients differed between sites (P <0.001) for all nutrients but did not differ by species.

	KB n=17	DK n= 20	VGK n= 20	Site	Species	Site × Species	Fynbos	Renosterveld
Total N (mg kg^{-1})	455 ± 54^{a}	906 ± 81^{b}	1443 ± 124^{c}	45.8 ***	0.6 *NS*	0.1 *NS*	1700 ± 100	1200 ± 100
Total P (mg kg^{-1})	959 ± 13^{a}	284 ± 7^{b}	507 ± 23^{c}	364 ***	2.7 *NS*	1.4 *NS*	249 ± 10	279 ± 45
Available P (Bray II; mg kg^{-1})	11.5 ± 0.5^{b}	8.0 ± 0.5^{a}	10.2 ± 0.8^{ab}	8.5 ***	0.4 *NS*	0.6 *NS*	13.3 ± 1.7	13.3 ± 1.2
K (mg kg^{-1})	19 ± 1^{a}	45 ± 6^{b}	73 ± 6^{c}	58.1 ***	0.09 *NS*	1.2 *NS*	84 ± 5	143 ± 16
Na ($cmol^{+}$ kg^{-1})	0.09 ± 0.003^{a}	0.25 ± 0.03^{b}	0.35 ± 0.3^{c}	44.9 ***	0.9 *NS*	1.0 *NS*	0.22 ± 0.03	0.35 ± 0.04
Ca ($cmol^{+}$ kg^{-1})	12.4 ± 0.27^{a}	15.5 ± 0.35^{b}	16.7 ± 0.59^{b}	31.7 ***	0.4 *NS*	1.5 *NS*	3.7 ± 0.3	5.2 ± 1
Mg ($cmol^{+}$ kg^{-1})	0.37 ± 0.02^{a}	0.99 ± 0.19^{b}	1.6 ± 0.16^{c}	64.7 ***	1.4 *NS*	2.5 *NS*	1.3 ± 0.1	2.3 ± 0.4
$\delta^{15}N$ (‰)	1.4 ± 0.3 ^{a}	3.2 ± 0.2 ^{b}	4.9 ± 0.2 ^{c}	72.7 ***	7.2 ***	3.6 **	–	–

***** *P* <0.001, ** *P* < 0.01, * *P* <0.05 , *NS*: No significance**

5.4.2 Foliar nutrients

Foliar N concentrations of *A. cyclops* were *ca.* 30-50% higher than foliar N of the native species. The De Kelders site exhibited lower foliar N concentrations of species combined (9.2 ± 1.1 mg g^{-1}) compared to the Koeberg (11.8 ± 1.1 mg g^{-1}) and Vergaderingskop (11.1 ± 0.8 mg g^{-1}) sites. (Table 5.3, Fig. 5.1). Despite the significant site differences of the species combined (Table 5.3), foliar N concentrations within a species were similar across the three sites even though total soil N varied considerably between sites (Table 5.2).

Foliar P concentrations of *A. cyclops* were on the other hand, similar to the foliar P of native species. *Acacia cyclops* and S*. laevigata* showed slightly elevated foliar P concentrations at Vergaderingskop but these site-specific differences were only marginally significant (P = 0.06; Table 5.3). The De Kelders site exhibited lower foliar P concentrations of the species combined (1.9 ± 0.1 mg g^{-1}) than at the Koeberg (2.9 ± 0.2 mg g^{-1}) and Vergaderingskop (2.9 ± 0.2 mg g^{-1}) sites (Table 5.3, Fig. 5.1), which was congruent with the low total and available soil P at De Kelders compared to the other two sites (Table 5.2).

Foliar N:P ratios were *ca*. 30-50% higher in *A. cyclops* than in the native species (Table 5.3, Fig. 5.1). The higher N:P ratios in *A. cyclops* are more than likely attributable to differences in foliar N, since *A. cyclops* foliar P did not differ to that of the native species. Foliar N:P of the species combined did not differ across the sites (Koeberg = 4.9 ± 0.4, De Kelders = 4.2 ± 0.4, Vergaderingskop = 4.3 ± 0.3; Table 5.3, Fig. 5.1).

Table 5.3. Results of two-way ANOVAs for foliar N and P (mg g^{-1}), foliar N:P ratios, $\delta^{15}N$ (‰), $\delta^{13}C$ (‰) photosynthetic nitrogen use efficiency (PNUE; μmol CO_2 kg^{-1} N s^{-1}) and photosynthetic phosphorus use efficiency (PPUE; μmol CO_2 kg^{-1} P s^{-1}). *F*-values and significance are presented for the effects of species and site. Significant results are bolded. Data were log transformed if assumptions of normality were not met.

	Species		Site		Species × site	
	F	*P*	*F*	*P*	*F*	*P*
Foliar N	**91.9**	**< 0.001**	**20.4**	**< 0.001**	2.3	0.07
Foliar P	**4.1**	**0.01**	**23.7**	**< 0.001**	2.4	0.06
Foliar N:P	**28.4**	**< 0.001**	81.6	0.2	2.3	0.06
$\delta^{15}N$	**9.4**	**< 0.001**	1.4	0.3	**2.5**	**0.05**
$\delta^{13}C$	**10.2**	**< 0.001**	2.2	0.1	1.3	0.27
PNUE	11.4	**< 0.001**	15.0	**< 0.001**	**3.7**	**0.009**
PPUE	37.8	**< 0.001**	17.4	**< 0.001**	8.4	**< 0.001**

Table 5.4. Results of three-way ANOVA for photosynthetic rate (A; μmol m^{-2} s^{-1}), transpiration rate (E; mmol m^{-2} s^{-1}), water use efficiency (WUE; μmol $mmol^{-1}$), and δD (‰). *F*-values and significance are presented for the effects of species, season and site. Significant results are bolded. Data were log transformed before analysis if assumptions of normality were not met.

	A		E		WUE		δD	
	F	*P*	*F*	*P*	*F*	*P*	*F*	*P*
Species	**69.3**	**< 0.001**	**25.0**	**< 0.001**	**4.7**	**0.004**	**14.2**	**< 0.001**
Season	**10.5**	**0.002**	**29.8**	**< 0.001**	**7.2**	**0.009**	0.06	0.8
Site	**3.4**	**0.04**	9.1	**< 0.001**	**4.7**	**0.01**	**11.7**	**< 0.001**
Species × season	**5.0**	**0.003**	3.2	0.3	0.5	0.6	0.8	0.5
Species × site	**3.7**	**0.005**	5.1	**< 0.001**	**2.9**	**0.02**	1.7	0.2
Season × site	**10.3**	**< 0.001**	**10.9**	**< 0.001**	**29.4**	**< 0.001**	**34.9**	**< 0.001**
Season × site × species	2.0	0.9	1.8	0.1	0.8	0.5	2.1	0.1

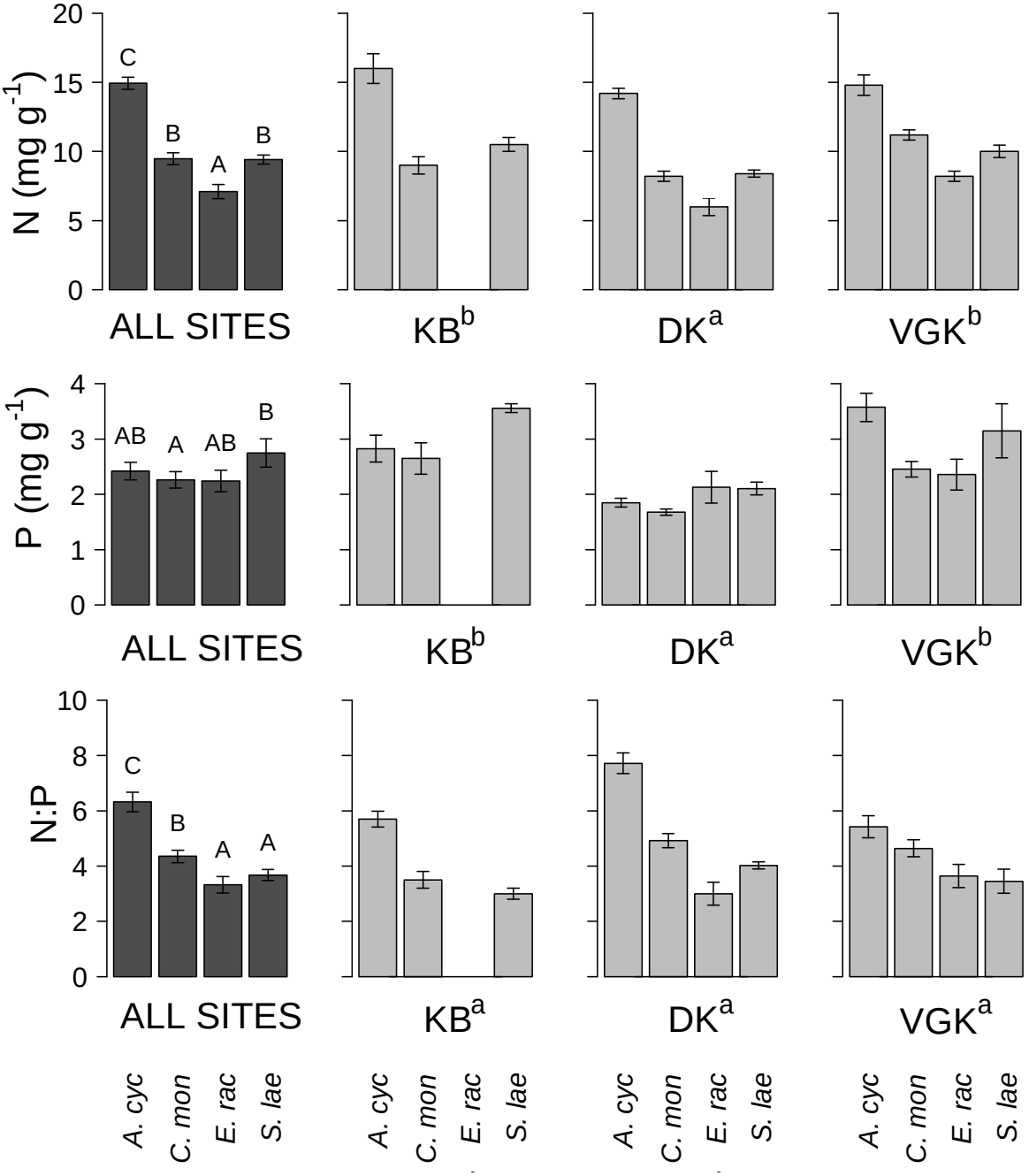

Figure. 5.1. Foliar N and P concentrations and N:P ratios of the invasive *A. cyclops* and the three native species: *C. monilifera*, *E. racemosa* and *S. laevigata* across all sites and at each site (KB: Koeberg, DK: De Kelders and VGK: Vergaderingskop). Bars indicate mean ± SE. Significant differences ($P < 0.05$) between species are indicated with uppercase lettering and between sites are indicated with lowercase lettering as determined from Tukey's post-hoc tests.

Foliar $\delta^{15}N$ varied between species differently amongst sites (Table 5.3). At Koeberg and De Kelders, $\delta^{15}N$ of *A. cyclops* (between -2 and -4) was more negative than $\delta^{15}N$ of the native species (between 1 and -1). At Vergaderingskop, however, *S. laevigata* and *A. cyclops* both exhibited equally more negative $\delta^{15}N$ (*ca.* -2) values than the other two native species (between 0 and 1; Table 5.3, Fig 5.2).

Foliar $\delta^{13}C$ varied significantly between species, with *A. cyclops* exhibiting higher $\delta^{13}C$ only in comparison to *C. monilifera* (Table 5.3, Fig 5.2). This was less pronounced at Vergaderingskop although this site-specific difference was not significant ($P = 0.27$). Foliar $\delta^{13}C$ of the species combined did not differ across the sites (Table 5.3, Fig. 5.2).

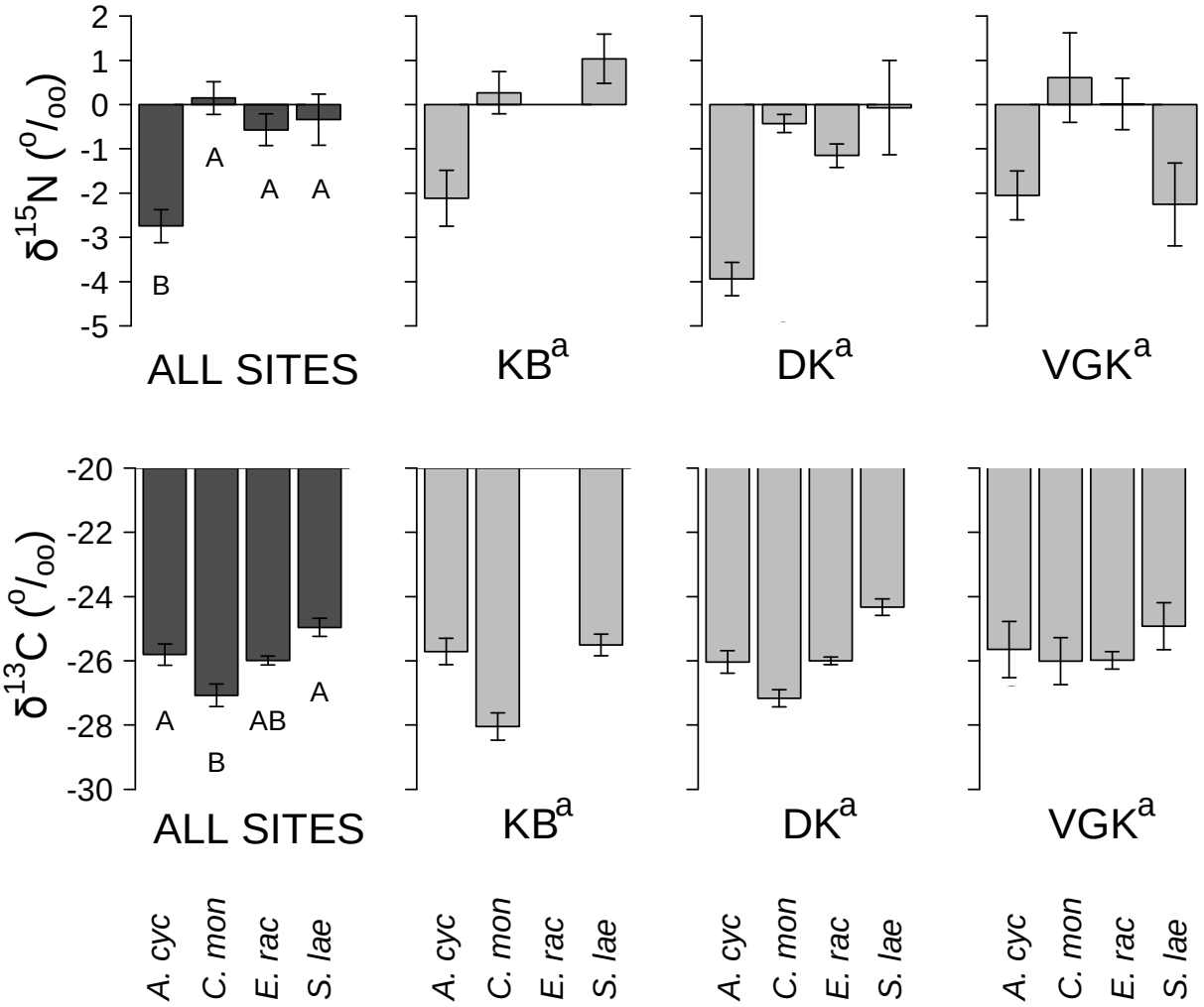

Figure 5.2. Foliar $\delta^{15}N$ and $\delta^{13}C$ of the invasive *A. cyclops* and the three native species: *C. monilifera*, *E. racemosa* and *S. laevigata* across all sites and at each site (KB: Koeberg, DK: De Kelders and VGK: Vergaderingskop). Bars indicate mean ± SE. Significant differences ($P < 0.05$) between species are indicated with uppercase lettering and between sites are indicated with lowercase lettering as determined from Tukey's post-hoc tests.

5.4.3. Gaseous exchange measurements

Photosynthetic rate (*A*) and transpiration (*E*) differed between species, but this varied according to season (Table 5.4). In summer, *A. cyclops* exhibited higher *A* than native species but in winter, *A* of *A. cyclops* and *C. monilifera* were similar and both greater than the other two native species (Fig 5.3). Transpiration in the summer was similar between *A. cyclops* and *C. monilifera* but greater than that of *E. racemosa* and *S.* laevigata (Fig. 5.3), whereas in winter, *A. cyclops* had similar transpiration to *C. monilifera* and *S. laevigata* but greater than *E. racemosa* (Fig. 5.3).

A. cyclops did not have consistently greater resource use efficiencies than the native species. Resource use efficiencies of *A. cyclops* were, however, never lower than the native species. In summer, *A. cyclops* only had WUEs higher only than that of *E. racemosa* and in winter WUE did not differ between species (Table 5.4, Fig. 5.3). Photosynthetic nitrogen and phosphorus use efficiency (PNUE and PPUE) differed between species differently at each site (Table 5.3). At Koeberg, PNUE of *A. cyclops* was similar to *C. monilifera* and *ca.* 30% greater than *S. laevigata.* At De Kelders, PNUE of *A. cyclops* was *ca.* 50% greater than *E. racemosa*, similar to *S. laevigata* and *ca.* 50% lower than *C. monilifera* and at Vergaderingskop all species tended to exhibit similar PNUEs (Fig. 5.4). PPUEs were on average similar between *A. cyclops* and *C. monilifera*, which were greater than that of *E. racemosa* and *S. laevigata*, although these differences were less pronounced at Vergaderingskop than at the other two sites (Fig. 5.4)

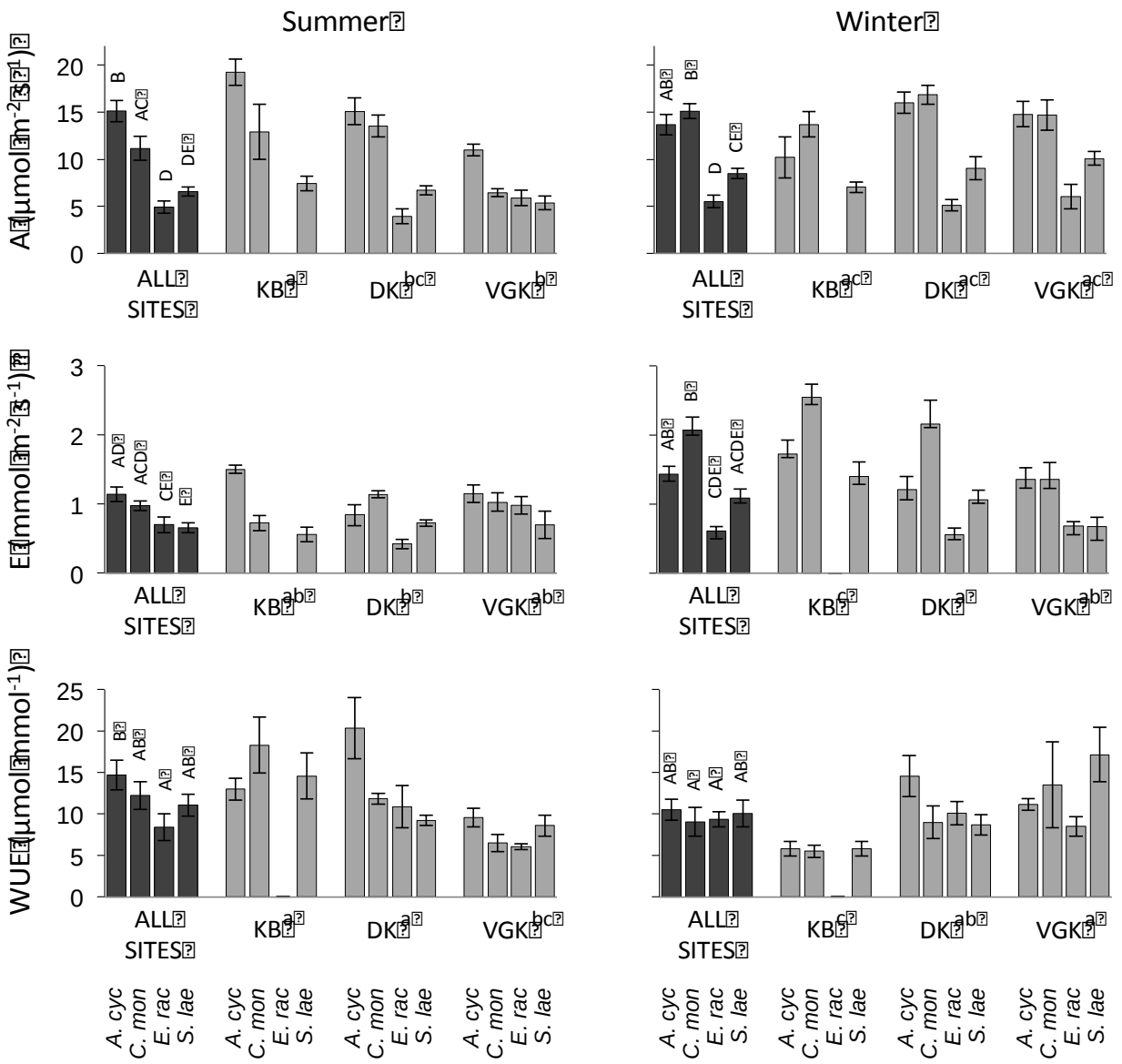

Figure 5.3. Photosynthetic rate (*A*), transpiration (*E*) and water use efficiency (WUE) in summer and winter of the invasive species, *A. cyclops,* in comparison to the native species: *C. monilifera*, *E. racemosa* and *S. laevigata* across all sites and at each site (KB: Koeberg, DK: De Kelders and VGK: Vergaderingskop). Bars indicate mean ± SE. Significant differences ($P < 0.05$) between species are indicated with uppercase lettering and between sites are indicated with lowercase lettering as determined from Tukey's post-hoc tests.

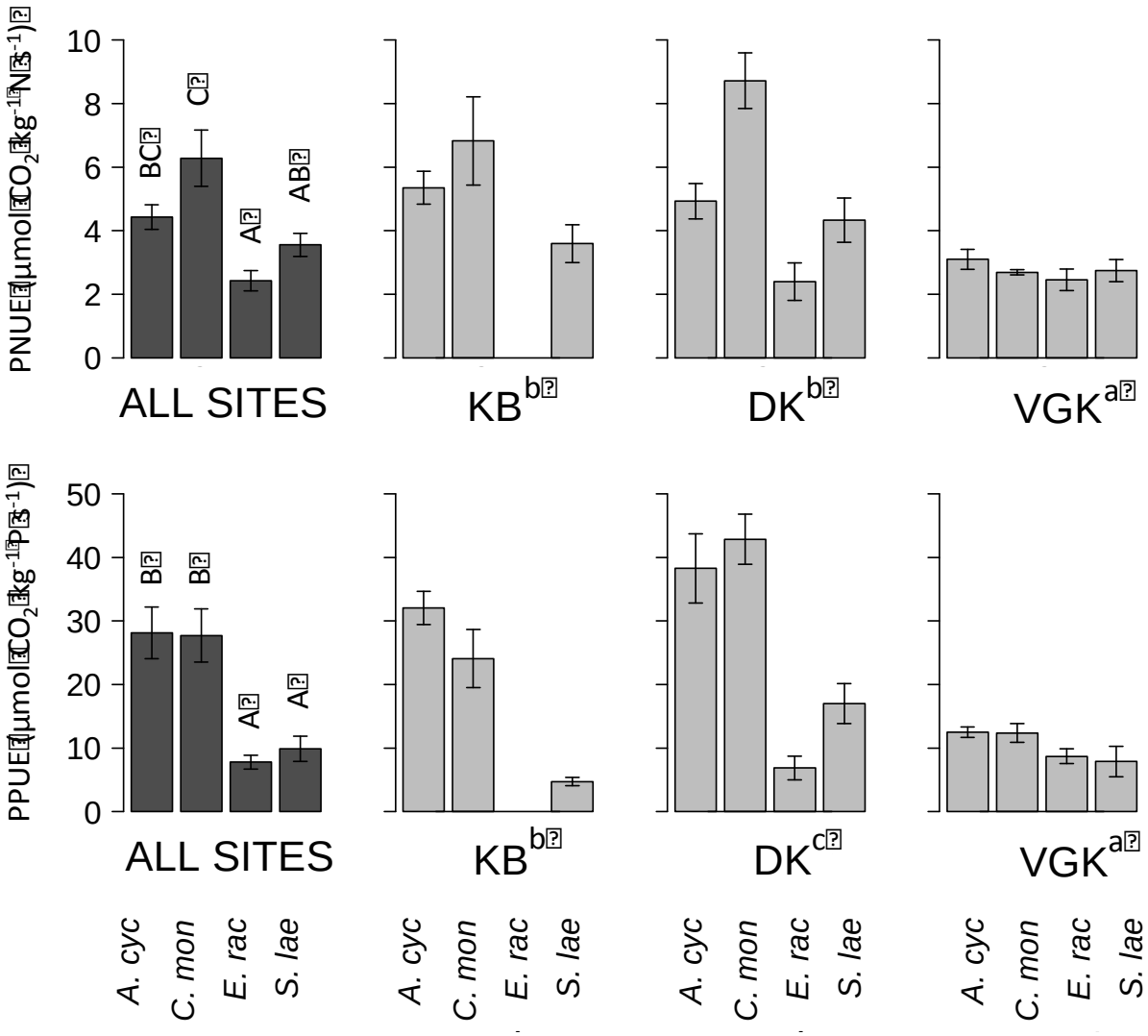

Figure 5.4. Photosynthetic nitrogen use efficiency (PNUE) and photosynthetic phosphorus use efficiency (PPUE) from the summer season of the invasive species, *A. cyclops,* in comparison to the native species: *C. monilifera*, *E. racemosa* and *S. laevigata* across all sites and at each site (KB: Koeberg, DK: De Kelders and VGK: Vergaderingskop). Bars indicate mean ± SE. Significant differences ($P < 0.05$) between species are indicated with uppercase lettering and between sites are indicated with lowercase lettering as determined from Tukey's post-hoc tests.

5.4.4. Rooting depth

Stem xylem water δD was used to estimate the relative differences in soil depth from which species are acquiring water resources. Overall, *A. cyclops* had more negative δD than the native species (Table 5.4, Table 5.5), which suggest that it was acquiring water form deeper in the soil profile in comparison to the native species.

Table 5.5. Stem xylem δD (mean ± SE)of the invasive *A. cyclops* compared to the three native species: *C. monilifera*, *E. racemosa* and *S. laevigata* in summer and winter across all sites and at each site: Koeberg, De Kelders and Vergaderingskop. Significant differences ($P < 0.05$) between species are indicated with uppercase lettering and between sites are indicated with lowercase lettering as determined from Tukey's post-hoc tests.

Species	Koeberg		De Kelders		Vergaderingskop		All
	Summer	Winter	Summer	Winter	Summer	Winter	
A. cyc	-40 ± 4	-36 ± 1	-46 ± 3	-50 ± 1	-44 ± 1	–	**-43 ± 2^{C}**
C. mon	-35 ± 0	-23 ± 3	-26 ± 3	-36 ± 2	-25 ± 4	–	**-29 ± 2^{A}**
E. rac			-32 ± 4	-46 ± 6	-31 ± 4	–	**-37 ± 3^{B}**
S. lae	-43 ± 3	-26 ± 3	-36 ± 3	-44 ± 2	-26 ± 4	–	**-35 ± 2^{B}**
All species	**-40 ± 2^{bc}**	**-28 ± 2^{a}**	**-35 ± 3^{ab}**	**-44 ± 2^{c}**	**-30 ± 3^{a}**	–	

5.5 Discussion

Acacia cyclops exhibited traits congruent with greater resource acquisition strategies, while using resources just as efficiently as the adapted native species. This differed to recent research, which correlated performance of invasive plant species with traits of greater resource use efficiency in resource limited environments (Funk and Vitousek 2007, Matzek 2011 reviewed in Funk 2013) suggesting this may be context, species or even life stage specific.

Acacia cyclops and *C. monilifera* exhibited higher photosynthetic rates than the other two native species in the wet winter season. Similar wet season results were previously reported for the closely related invasive *A. saligna* and *A. longifolia* species, which along with *C. monilifera* exhibited greater winter photosynthetic rates than three other native species in the abiotically similar fynbos vegetation ecosystem of the CFR (Kraaij and Cramer 1999). In the drier summer season, however, *A. cyclops* exhibited higher photosynthetic rates than all the native species. The ability for *A. cyclops* to sustain high photosynthetic activity in both seasons may provide the invasive species with a performance advantage over the native species especially under periods of high growth demand.

Transpiration of *A. cyclops* did not differ across seasons. This suggests that the ability for *A. cyclops* to maintain high photosynthetic activity was likely due to sustained water acquisition even in the dry summer season rather than due to increased WUE, which did not differ between seasons. This may indicate that *A. cyclops* is able to acquire water resources not available to native species, particularly in the dry summer season. This notion is supported by the foliar δD results, which suggests that *A. cyclops* may acquire

water resources from depths deeper than that of the native species. While these data do not allow us to determine the specific depths to which *A. cyclops* roots may be penetrating, they do suggest that rooting systems may be deep enough to acquire water resources not available to the native species. This would provide *A. cyclops* with a resource acquisition advantage, especially under water-limited conditions. Deeper rooting of invasive Australian Acacias has previously been suggested although data is scarce for mature trees due to difficulties associated with assessing mature rooting profiles (Morris *et al.* 2011).

WUE did not differ markedly between *A. cyclops* and native species in either instantaneous WUE measures or $\delta^{13}C$ values, generally correlated with long term WUE (Farquhar *et al.* 1982). These results are similar to those found for other invasive Australian *Acacia* species and other invasive species in general, which exhibited little difference in measures of WUE between invasive and native species (reviewed in Funk 2013, Morris *et al.* 2011). While *A. cyclops* did not have greater WUE than native species, WUE of *A. cyclops* was also not lower than that of the native species. Australian *Acacia* invasions in South Africa, are known to reduce surface water runoff, impacting ecosystem services and water security (Görgens and van Wilgen 2004, De Lange and van Wilgen 2010). Results from my study indicate these reductions are unlikely to be due to low water use efficiencies of *A. cyclops.* Instead, the water usage of this (and possibly other Australian *Acacia* species) may be more attributable to the larger canopy biomass, functioning at consistently higher gaseous exchange rates, especially in the drier summer seasons.

Significantly greater foliar N of *A. cyclops* at all sites, despite strong differences in soil N concentrations may indicate that *A. cyclops* is better able to acquire nitrogen than the native species. Since invasive Australian *Acacias*, including *A. cyclops*, are well documented

to be strong N_2-fixers in invaded ranges Marchante *et al.* 2008, reviewed in Morris *et al.* 2011, Rodríguez-Echeverría *et al.* 2009), increased foliar N concentrations may be due to N_2-fixation.

Foliar $\delta^{15}N$ values close to zero or below (due to fragmentation during fixation) are generally indicative of the occurrence of N_2-fixation (Lajtha and Marshall 1994). While foliar $\delta^{15}N$ results of *A. cyclops* (-2.00 to -4.00 ‰) are more negative than would generally be expected, similar values (0.00 to -2.00 ‰) have previously been reported for *A. cyclops* plants grown in N-free hydroponic soils (Stock *et al*. 1995) and in *A. cyclops* seedlings when growing in low nutrient environments (-2.00 to -4.00 ‰; Chapter 2). Thus the low foliar $\delta^{15}N$ values are likely to indicate N_2-fixation.

Contrary to findings of other studies that report invasive species in resource limited environments to exhibit greater PNUE than native species (reviewed in Funk 2013), *A. cyclops* did not exhibit greater PNUE than native species. This is congruent with assessments of PNUE in other invasive Australian Acacias: *A. saligna, A. longifolia,* and *A. melanoxylon,* which also found similar PNUE between the invasive species and native comparisons (Godoy *et al*. 2011, Kraaij and Cramer 1999). This may potentially be due to the N_2-fixation abilities of *A. cyclops* ameliorating N-limitations of the plants.

In contrast, PPUE of *A. cyclops* was greater than that of *E. racemosa* and *S. laevigata* but similar to *C. monilifera*. While little is known of the PPUE of other invasive Australian *Acacias* or of the mechanisms that might produce higher PPUE, Lambers *et al*. 2012) found that several woody Australian species, adapted to the P-impoverished soils of south-western Australia, increase PPUE by substituting phospholipids with galactolipids and sulfolipids. If similar mechanisms were occurring in invasive Australian *Acacias*, this might

allow for the scarce phosphorus resources to be used in ecophysiological processes other than carbon assimilation such as N_2-fixation, which would provide an advantage for the invasive N-fixing species. Thus, further examination of the existence and mechanisms of PPUE in invasive Australian *Acacias* could be useful in elucidating mechanisms and advantages of PPUE in low phosphorus environments such as the CFR.

In conclusion, *A. cyclops* was able to maintain consistently high photosynthetic rates over both seasons, distinguishing performance of the invader in comparison to the native species. While it is unlikely that this performance is associated with greater resource use efficiency than the native species, results suggest that N_2-fixation and deep rooting potential may enable *A. cyclops* to overcome resource limitations imposed by the abiotic constraints of Mediterranean-climate shrublands and facilitating successful invasion in resource limited environments.

CHAPTER 6
CONCLUSION

Limiting the spread of tree invasions into untransformed native ecosystems is becoming an increasing priority for long term alien plant management (Caplat *et al*. 2012; Hulme 2012; Moore *et al.* 2011; van Wilgen *et al.* 2011). The goal of my dissertation was to investigate the barriers imposed by native ecosystems to demographic life stages of an invasive tree. Specifically, I explored how the invasive tree species, *A. cyclops*, was constrained by native Mediterranean-climate shrublands and whether traits of the invader may aid in navigating barriers to establishment, growth and spread imposed by native ecosystems.

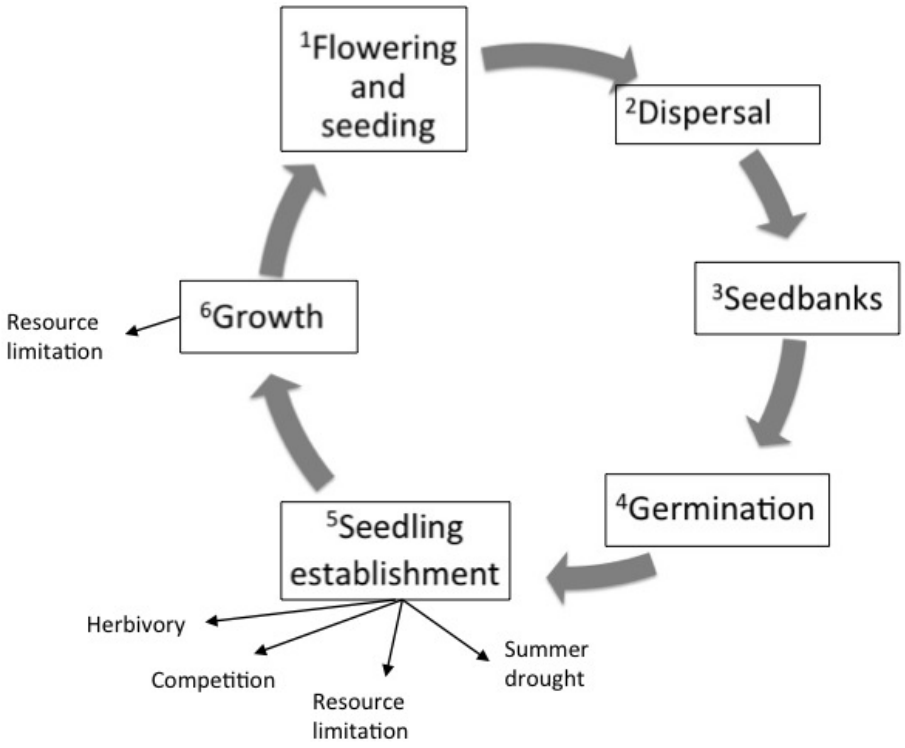

Figure 6.1. Barriers to seedling establishment and growth of the invasive tree, *A. cyclops* imposed by biotic and abiotic components of Mediterranean-climate shrublands.

My results indicate that early life stages of a tree invader are most susceptible to the biotic components of ecosystem resistance since both seedling herbivory and native plant competition reduced early establishment of *A. cyclops* seedlings transplanted into native strandveld (Fig. 6.1). Herbivory is likely to be reduced when seedlings establish in aboveground vegetation gaps, whereas plant competition is likely to be reduced in "root" gaps. Once seedlings are able to establish, abiotic constraints are likely to limit further growth and invasion success. Key ecophysiological traits of the invader are likely to aid in navigation of abiotic resource constraints in the seedling establishment and growth phases (Fig. 6.1). These include rapid and substantial allocation to root biomass, N_2-fixation, and heteroblasty.

These findings provide evidence for the importance of native ecosystems for biotic resistance to invasion. Results may be useful in predicting invasion risks to different native communities, identifying targets for ecosystem restoration and informing decisions on management and clearing operations so as to maximize ecosystem resistance.

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APPENDIX

Table A1, Chapter 3: Vegetation variables (mean ± SE) measured at each study site. *P* values are provided for significant differences between vegetation types, determined with non-parametric two-tailed t-tests with a *d.f.* =3

	Koeberg	Vergaderingskop	*P*
Canopy cover (%)	1.3 ± 0.9	1.7 ± 0.3	0.75
Height (m)	1.2 ± 0.1	1.9 ± 0.1	**0.005**
Sand cover (%)	55.2 ± 3.7	6 ± 0.5	**0.004**
Herbaceous cover (%)	10.2 ± 2.3	31.1 ± 1.8	**0.002**
Litter cover (%)	34.6 ± 5.2	62.9 ± 1.6	**0.02**
Vertical vegetation density: 0-0.5 (m^2 m^{-3})	0.58 ± 0.14	1.05 ± 0.28	0.23
Vertical vegetation density: 0.5-1 (m^2 m^{-3})	0.33 ± 0.07	0.49 ± 0.06	0.13
Vertical vegetation density: 1-1.5 (m^2 m^{-3})	0.1 ± 0.03	0.31 ± 0.03	**0.006**
Vertical vegetation density: 1.5-2 (m^2 m^{-3})	0.08 ± 0.04	0.13 ± 0.02	0.42
Native woody species richness	4.7 ± 0.9	11.7 ± 2.2	0.07
Native herbaceous species richness	8.7 ± 0.9	22.7 ± 3.5	0.05